

## **Increased alarm pheromone component is associated with *Nosema ceranae* infected honeybee colonies**

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### **Article citation details**

*R. Soc. open sci.* **8**: 210194.

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### **Review timeline**

Original submission: 3 February 2021

Revised submission: 9 April 2021

Final acceptance: 9 April 2021

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 with peer review.

## Review History

### RSOS-210194.R0 (Original submission)

#### Review form: Reviewer 1

**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

**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)**

The manuscript is now improved but they still need to be cautious with the interpretation of results in the abstract. In line 46, they mention this increase in alarm pheromone production might drive the killing of infected nestmates. There is no evidence for that. In addition, in the discussion they mention different outcomes for this pheromone change, such as more care, kill or quarantine. However, in the abstract they only cite the killing hypothesis. They should therefore either remove it from the abstract or fairly report all possible outcomes.

The following reference, where a change in CHCs was found upon *Nosema* infection, is missing. No change in behavioural interactions was found.

McDonnell, C.M., Alaux, C., Parrinello, H. et al. Ecto- and endoparasite induce similar chemical and brain neurogenomic responses in the honey bee (*Apis mellifera*). *BMC Ecol* 13, 25 (2013)

Line 174 : it is still not clear what is the amount of spores ; 3.99 millions? If yes, millions should be added. In addition, what do the numbers in parenthesis mean? 3.99 (547,045 spores/bee)? I am sorry but I am confused by how the number of spores are reported.

Line 219: "sometimes kill an *N. ceranae* infected individual", is there evidence for that? A reference?

**Decision letter (RSOS-210194.R0)**

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Mayack

On behalf of the Editors, we are pleased to inform you that your Manuscript RSOS-210194 "Increased alarm pheromone component is associated with *Nosema ceranae* infected honey bee colonies" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referees' reports. Please find the referees' comments along with any feedback from the Editors below my signature.

We invite you to respond to the comments and revise your manuscript. Below the referees' and Editors' comments (where applicable) we provide additional requirements. Final acceptance of your manuscript is dependent on these requirements being met. We provide guidance below to help you prepare your revision.

Please submit your revised manuscript and required files (see below) no later than 7 days from today's (ie 07-Apr-2021) date. Note: the ScholarOne system will 'lock' if submission of the revision is attempted 7 or more days after the deadline. If you do not think you will be able to meet this deadline please contact the editorial office immediately.

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submitted as part of our collaboration with the Royal Society of Chemistry (<https://royalsocietypublishing.org/rsos/chemistry>). Fee waivers are available but must be requested when you submit your revision (<https://royalsocietypublishing.org/rsos/waivers>).

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

Kind regards,  
Royal Society Open Science Editorial Office  
Royal Society Open Science  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

on behalf of Prof Kevin Padian (Subject Editor)  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

Editor comments:

Thanks for your efforts in revision. We are happy to accept your manuscript but we would like you to address specifically the comments of the reviewer in your final version before we can process it. Best wishes.

Reviewer comments to Author:

Reviewer: 1

Comments to the Author(s)

The manuscript is now improved but they still need to be cautious with the interpretation of results in the abstract. In line 46, they mention this increase in alarm pheromone production might drive the killing of infected nestmates. There is no evidence for that. In addition, in the discussion they mention different outcomes for this pheromone change, such as more care, kill or quarantine. However, in the abstract they only cite the killing hypothesis. They should therefore either remove it from the abstract or fairly report all possible outcomes.

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Line 219: "sometimes kill an *N. ceranae* infected individual", is there evidence for that? A reference?

===PREPARING YOUR MANUSCRIPT===

Your revised paper should include the changes requested by the referees and Editors of your manuscript. You should provide two versions of this manuscript and both versions must be provided in an editable format:

one version identifying all the changes that have been made (for instance, in coloured highlight, in bold text, or tracked changes);

a 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them. This version will be used for typesetting.

Please ensure that any equations included in the paper are editable text and not embedded images.

Please ensure that you include an acknowledgements' section before your reference list/bibliography. This should acknowledge anyone who assisted with your work, but does not qualify as an author per the guidelines at <https://royalsociety.org/journals/ethics-policies/openness/>.

While not essential, it will speed up the preparation of your manuscript proof if you format your references/bibliography in Vancouver style (please see <https://royalsociety.org/journals/authors/author-guidelines/#formatting>). You should include DOIs for as many of the references as possible.

If you have been asked to revise the written English in your submission as a condition of publication, you must do so, and you are expected to provide evidence that you have received language editing support. The journal would prefer that you use a professional language editing service and provide a certificate of editing, but a signed letter from a colleague who is a native speaker of English is acceptable. Note the journal has arranged a number of discounts for authors using professional language editing services (<https://royalsociety.org/journals/authors/benefits/language-editing/>).

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Attach your point-by-point response to referees and Editors at Step 1 'View and respond to decision letter'. This document should be uploaded in an editable file type (.doc or .docx are preferred). This is essential.

Please ensure that you include a summary of your paper at Step 2 'Type, Title, & Abstract'. This should be no more than 100 words to explain to a non-scientific audience the key findings of your research. This will be included in a weekly highlights email circulated by the Royal Society press office to national UK, international, and scientific news outlets to promote your work.

At Step 3 'File upload' you should include the following files:

-- Your revised manuscript in editable file format (.doc, .docx, or .tex preferred). You should upload two versions:

- 1) One version identifying all the changes that have been made (for instance, in coloured highlight, in bold text, or tracked changes);
- 2) A 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them.

-- An individual file of each figure (EPS or print-quality PDF preferred [either format should be produced directly from original creation package], or original software format).

-- An editable file of each table (.doc, .docx, .xls, .xlsx, or .csv).

-- An editable file of all figure and table captions.

Note: you may upload the figure, table, and caption files in a single Zip folder.

-- Any electronic supplementary material (ESM).

-- If you are requesting a discretionary waiver for the article processing charge, the waiver form must be included at this step.

- If you are providing image files for potential cover images, please upload these at this step, and inform the editorial office you have done so. You must hold the copyright to any image provided.
- A copy of your point-by-point response to referees and Editors. This will expedite the preparation of your proof.

At Step 6 'Details & comments', you should review and respond to the queries on the electronic submission form. In particular, we would ask that you do the following:

- Ensure that your data access statement meets the requirements at <https://royalsociety.org/journals/authors/author-guidelines/#data>. You should ensure that you cite the dataset in your reference list. If you have deposited data etc in the Dryad repository, please only include the 'For publication' link at this stage. You should remove the 'For review' link.
- If you are requesting an article processing charge waiver, you must select the relevant waiver option (if requesting a discretionary waiver, the form should have been uploaded at Step 3 'File upload' above).
- If you have uploaded ESM files, please ensure you follow the guidance at <https://royalsociety.org/journals/authors/author-guidelines/#supplementary-material> to include a suitable title and informative caption. An example of appropriate titling and captioning may be found at [https://figshare.com/articles/Table\\_S2\\_from\\_Is\\_there\\_a\\_trade-off\\_between\\_peak\\_performance\\_and\\_performance\\_breadth\\_across\\_temperatures\\_for\\_aerobic\\_scope\\_in\\_teleost\\_fishes\\_/3843624](https://figshare.com/articles/Table_S2_from_Is_there_a_trade-off_between_peak_performance_and_performance_breadth_across_temperatures_for_aerobic_scope_in_teleost_fishes_/3843624).

At Step 7 'Review & submit', you must view the PDF proof of the manuscript before you will be able to submit the revision. Note: if any parts of the electronic submission form have not been completed, these will be noted by red message boxes.

## Author's Response to Decision Letter for (RSOS-210194.R0)

See Appendix A.

## Decision letter (RSOS-210194.R1)

We hope you are keeping well at this difficult and unusual time. We continue to value your support of the journal in these challenging circumstances. If Royal Society Open Science can assist you at all, please don't hesitate to let us know at the email address below.

Dear Dr Mayack,

I am pleased to inform you that your manuscript entitled "Increased alarm pheromone component is associated with *Nosema ceranae* infected honey bee colonies" is now accepted for publication in Royal Society Open Science.

If you have not already done so, please remember to make any data sets or code libraries 'live' prior to publication, and update any links as needed when you receive a proof to check - for instance, from a private 'for review' URL to a publicly accessible 'for publication' URL. It is good practice to also add data sets, code and other digital materials to your reference list.

You can expect to receive a proof of your article in the near future. Please contact the editorial office ([openscience@royalsociety.org](mailto:openscience@royalsociety.org)) and the production office ([openscience\\_proofs@royalsociety.org](mailto:openscience_proofs@royalsociety.org)) to let us know if you are likely to be away from e-mail contact -- if you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal. Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication.

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On behalf of the Editors of Royal Society Open Science, thank you for your support of the journal and we look forward to your continued contributions to Royal Society Open Science.

Best regards,  
Lianne Parkhouse  
Editorial Coordinator  
Royal Society Open Science  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

on behalf of Professor Kevin Padian (Subject Editor)  
[openscience@royalsociety.org](mailto:openscience@royalsociety.org)

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# Appendix A

## Response to Reviewer Comments

Dear Prof Kevin Padian and reviewer,

We would like to thank you for the detailed comments and feedback from the Royal Society Open journal submission. We realize that the reviewer process is voluntary and appreciate the time you have taken to review this manuscript.

We have made alterations to the abstract, results, discussion and added reference as suggested. With all of these comments addressed we now feel that our manuscript is improved. Below you will find a point by point response for the remaining issues that have been raised in italicized text. We look forward to hearing from you once again and are pleased to hear that the paper has been accepted for publication.

Sincerely,

Christopher Mayack

Reviewer comments to Author:

Reviewer: 1

Comments to the Author(s)

The manuscript is now improved but they still need to be cautious with the interpretation of results in the abstract. In line 46, they mention this increase in alarm pheromone production might drive the killing of infected nestmates. There is no evidence for that. In addition, in the discussion they mention different outcomes for this pheromone change, such as more care, kill or quarantine. However, in the abstract they only cite the killing hypothesis. They should therefore either remove it from the abstract or fairly report all possible outcomes.

*We agree with the reviewer that to be fair all possible outcomes should be stated in the abstract. We have now included the other possible outcomes in the abstract.*

The following reference, where a change in CHCs was found upon Nosema infection, is missing. No change in behavioural interactions was found.

McDonnell, C.M., Alaux, C., Parrinello, H. et al. Ecto- and endoparasite induce similar chemical and brain neurogenomic responses in the honey bee (*Apis mellifera*). BMC Ecol 13, 25 (2013)

*Thanks for pointing this out, we have now added this reference.*

Line 174 : it is still not clear what is the amount of spores ; 3.99 millions? If yes, millions should be added. In addition, what do the numbers in parenthesis mean? 3.99 (547,045 spores/bee)? I am sorry but I am confused by how the number of spores are reported.

*Now we understand where the confusion is coming from and this is because we performed a semi-quantitative analysis and the number refers to a relative quantification of the amount of spores with a unitless scale. At the same time, we report the spore numbers based on a standard curve generated from a previous study. To avoid confusion, we have removed the less informative semi-quantitative number.*

Line 219: “sometimes kill an *N. ceranae* infected individual”, is there evidence for that? A reference?

*Yes, there is evidence in the article that is cited in the manuscript which can also be found below...*

[33] Biganski, S., Kurze, C., Müller, M.Y. & Moritz, R.F.A. 2018 Social response of healthy honeybees towards *Nosema ceranae*-infected workers: care or kill? *Apidologie* **49**, 325-334. (doi:10.1007/s13592-017-0557-8).