PCI Ecology Response to Reviewers Round #2

Dear Jessie Abbate,

Your article, entitled **Pathogen community composition and co-infection patterns in a wild community of rodents**, has now been reviewed. The referees' comments and the recommender's decision are shown below. As you can see, the recommender found your article very interesting but suggests certain revisions.

We shall, in principle, be happy to recommend your article as soon as it has been revised in response to the points raised by the referees.

When revising your article, we remind you that:

1) Data must be available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad (to pay) or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data;

Done.

2) Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) must be available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused;

Done.

3) Details on experimental procedures must be available to readers in the text or as appendices;

Done.

4) Authors must have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this article declare that they have no financial conflict of interest with the content of this article.";

Done.

5) This disclosure has to be completed by a sentence indicating, if appropriate, that some of the authors are PCI recommenders: "XY is one of the PCIEcology recommenders.".

Done.

When your revised article is ready, please:

1) Upload the new version of your manuscript onto your favorite open archive;

Done. https://www.biorxiv.org/content/10.1101/2020.02.09.940494v3

2) Follow this link https://ecology.peercommunityin.org/user/my_articles or logging onto the PCIEcology website and go to 'For Contributors -> Your submitted preprints' in the top menu;

3) Make your changes to the title, summary, link to the article (or its DOI) and keywords if necessary by clicking on the 'Edit Article' button. If not already done, you also need to upload a picture or an illustration for which you own the rights – this picture will be used to illustrate your article, if recommended;

We tried to upload the most hi-fi image the system would accept, with limited success. Very buggy.

4) Write, copy/paste or upload (as a PDF file) your reply to the recommender's and reviewers' comments by clicking on the 'Write, edit or upload your reply to recommender' button. You can also upload (as a PDF file) a revised version of your article, with the modifications indicated in TrackChanges mode;

Done.

5) When you are ready to submit your new version, click on the 'Save & submit your reply' button.

Done.

Once the recommender has read the revised version, he/she may decide to recommend it directly, in which case the editorial correspondence (reviews, recommender's decisions, authors' replies) and a recommendation text will be published by PCIEcology under the license CC-BY-ND.

Round #2

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by Francois Massol, 2021-03-30 17:57
Manuscript: **https://doi.org/10.1101/2020.02.09.940494** version v1

Almost ready to recommend

Dear authors,

thanks a lot for the thorough revisions you completed. As you will read, the two reviewers (one from last round of reviews, the other new) much appreciated the current version. I agree with them and I think that after some minor revisions (i.e. considering the points raised by R. Pigeault), your paper will be ready to be recommended.

Excellent, Thank you.

I personally thank you for comparing the drop1 ANOVA tests with the model comparison procedure. However, from the results you show, I would really recommend using model comparisons (e.g. replacing drop1 tables by AICc tables of models or weight tables of variable importances) rather than drop1. From a purely statistical viewpoint, the comparisons that drop1 is making are not very relevant because they are grounded in the assumption that almost all tested variables should be included in the model. This can lead to spurious estimates of model coefficients, even if the “best model” and the drop1 tables seem to agree.

We have proposed a compromise between these two statistical philosophies. In the current version of the manuscript, we have included a model selection step that reduces the full model to the ‘best model including all pathogens of interest’ on which we then use analysis of deviance (drop1 function) to evaluate statistical significance (Lines 361-367). Given the large number of competing models, we consider this approach to provide the most tractable results for *a priori* tests while reducing the issue of spurious estimates. What we noticed is that the results are largely the same, and we’ve presented the results of the updated methods. Since *all* of our results represent hypotheses that require further exploration, we are comfortable with making this compromise and presenting the modified results.

I hope I will soon read the revision of this excellent paper.

Apologies for the “soon” part. This submission and these revisions in particular fell victim to the pandemic and a comedy of other unlucky circumstances. But we feel its relevance remains strong.

Sincerely,

François Massol

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Additional comments from the Managing board

Mandatory modifications
1- Please make sure that:
-Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data. -Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.

Done.

-Details on experimental procedures are available to readers in the text or as appendices. Include information about ethical approval for animal experimentation. Provide information about the compliance of their work with ethical standards of their national ethical committees and report the reference number of the ethical committee approval. If the study did not require ethical approval, include some sentences explaining why the approval was not needed.

Done.

-Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors
are PCI recommenders: "XXX is one of the PCI Ecology recommenders."

Done.

2- Please make the following changes:
-Add the following sentence in the acknowledgements: "Version 3 of this preprint has been peer-reviewed and recommended by Peer Community
In Ecology (https://doi.org/10.24072/pci.ecology.100071)"

Done.

-If you use bioRxiv to post your preprint, add this latter sentence also in the “revision summary” section of the deposit form of bioRxiv.
Note that this DOI is not the DOI of your article, but the DOI of the recommendation text. The DOI of your article remains unchanged.

Done.

3- If not yet done, please send us a picture for which you own the rights that could serve as a thumbnail/illustration for your article on the web site of PCI. It can be a figure of the article.

Done.

Optional instructions (we strongly advise you to follow them)
1- We suggest you to remove line numbering from the preprint and put the tables and figures within the text rather than at the end of your MS.

2- Then, we strongly advise you to use the PCI templates (word docx template or latex template) to format your preprint in a PCI style. Here is the links of the templates: https://peercommunityin.org/templates/
→ For word template:
Do not hesitate to modify the template as you want (and send it back to us if you made significant improvements).
-the text to be replaced by your own text starts with XXX, eg XXXXTitle of the article.
-XXXXthe "citeas" → Edeline, E. and Loeuille, N. (2021) Size-dependent eco-evolutionary feedbacks in fisheries. bioRxiv, 2020.04.03.022905, ver. 3 peer-reviewed and recommended by PCI Ecology. doi: https://doi.org/10.1101/2020.04.03.022905
-XXXXthe date of deposit in the preprint server → date of the deposit of the latest version
-XXXXthe surnames and names of the reviewers we sent you → Jean-François Arnoldi and an anonymous reviewer
-XXXXthedoiwesentyou → https://doi.org/10.24072/pci.ecology.100071
-XXXXthe surname and name of the recommender → Simon Blanchet
-In the acknowledgements, add this sentence → "Version 3 of this preprint has been peer-reviewed and recommended by Peer Community
In Ecology (https://doi.org/10.24072/pci.ecology.100071)"
-Please be careful to choose the badges “Open Code” and “Open Data” only if appropriate (in addition to the “Open Access” and “Open Peer-Review” badges).
→ For Latex and mode org templates:
Do not hesitate to modify the template as you want (and send it back to us if you made significant improvements).
-main.tex and sample.bib should be filled.
-in main.tex, the recommender’s name is "Simon Blanchet" and the reviewers’ names are Jean-François Arnoldi and an anonymous reviewer -In sample.bib, indicate the right

version of your preprint. It is version 3
-Preambuleecology.tex should be modified (comment lines 115, 119) to select badges. Please be careful to choose the badges “Open Code” and “Open Data” only if appropriate (in addition to the “Open Access” and “Open Peer-Review” badges).

We formatted after submitting to the preprint server. We’ve included the formatted version as PDF at the end of the Tracked Changes document submitted.

3- we suggest that you deposit a copy of your MS in zenodo.org and ask for its inclusion in the PCI community (“Communities” section in the deposit form). Indicate the current doi of your MS, if it already has one, in the “doi” section.

OK, but we will do this once the final version, including correct formatting, has been accepted. It already points to the current version from biorxiv.

Reviews

Reviewed by Adrian Diaz, 2021-03-29 18:03

Dear Recommender,

Authors have address my comments and suggestions in a satisfactory way. So, I have no further comments for this manuscript.

Thank you for taking the time to re-review our manuscript.

Reviewed by Romain Pigeault, 2021-03-17 09:25

Dear Recommender,

In this manuscript authors aimed to characterize the parasite community harbouring by a wild community of rodents in France.

By combining different detection methods (molecular characterisation for pathogenic bacteria and antibody tests for viruses) and extensive statistical analyses, they explored how extrinsic (e.g. habitat, age, sex) and intrinsic factors shaped parasite community. They highlighted that host species is the most important factor of parasite community composition. Authors also confirm some specific pattern in parasite interactions (positive association) already published in literature.

The topic covered in this article is very interesting and relevant. I must say that I really enjoyed reading this manuscript. Although the article is very dense, it is very well written

as well as very well structured, which makes it very enjoyable to read. The corrections/modifications made by the authors following the reviewers' comments seem to me to be quite appropriate. In reading the manuscript, I noted several limitations, related to the methodological and statistical approaches, but most of them are acknowledged and discussed by the authors. At this stage I have only a few minor comments and suggestions.

Super! We thank the reviewer for their input.

1) Although the authors state in the discussion that phylogeny has a minimal impact on pathogen diversity in rodent species, I would have been very curious to know the proportion of variance that is attributable to phylogeny and species identity respectively.

We agree that this is an interesting question, however, we feel that responding to it with the data we currently have may introduce biases because we did not design it to look at phylogenetic differences, thus we did not sample to include necessary balances in the representation of taxa. It is an excellent follow-up study question, particularly for a study with higher sample sizes.

2) As the authors also seem to have individual data in addition to sex and age ("precise body measurements" lines 160-162), it would also have been interesting to study the impact of these parameters on parasite richness and individual parasite community composition (e.g. Mangombi et al 2021 10.1371/journal.pone.0248244).

While we agree that this would have been super interesting, we were concerned that there was too much variation introduced by the methods, as body measurements needed for mass calculations such as length and weight were taken at the time of dissection and were likely *not* precise. This was a type-o and we have now changed it to correctly read *specific* body measurements (eg, size of gonads).

In addition, we wanted to focus on factors that were not likely *caused by* the pathogen community, but factors that influence the pathogen community. That said, the distinction is clearly not black-and-white, since modification of host condition or behavior can be a mechanism of pathogen-pathogen interaction. We hope future studies might address this interesting point. See Beldomenico et al. 2008 Proc Roy Soc B 10.1098/rspb.2008.0147

3) Line 31: It would be useful to add (in brackets) the extrinsic factors.

Done.

4) Line 83: it would be relevant to cite the recent review published by Karvonen et al., 2019 (10.1016/j.pt.2018.11.007)

Done.

5) Lines 83-89: Mentioning the impact of co-infections on the evolution of virulence (Alizon et al 2013, 10.1111/ele.12076) and the maintenance of parasite genetic variation (and thus the impact on host/parasite coevolution, Seppälä et al 2016, 10.1016/j.pt.2016.08.010)might be relevant here?

Done.

6) Lines 101-102: I don't understand what "strong ecological interactions" refers to (competition and facilitation?).

Yes. We have clarified. “Strong ecological interactions, such as facilitation and competition, have been shown in wild rodent populations among some…”

7) Line 158: Is there a particular reason why the sampling was done in autumn? In rodents, seasonal changes can cause qualitative and quantitative differences in the gut microbiota (Maurice et al 2015, 10.1038/ismej.2015.53) but also in the composition of pathogenic bacterial communities (e.g. Kleynhans et al 2018, 10.1016/j.meegid.2018.07.036, Villette et al 2020, 10.1038/s41598-020-66107-5). Perhaps this limitation could be briefly mentioned (e.g. lines 718-721).

We have added this limitation and some references in lines 936-938.

8) Line 160: Suggestion: use “age class” instead of “age”.

Done.

9) Line 169: How are the different habitats distributed in the two study sites?

The entire sampling method, including the distribution of habitats and sites, is thoroughly described in the study referenced in [previous] line 160 (Gotteland etal 2014).

10) Line 194: please add references.

Done. “Given that rare, low abundance taxa tend to show high dissimilarity between sample replicates (D. P. Smith & Peay, 2014), we assumed that only OTUs with at least 500 reads across all animals in the dataset were considered reliably detectable, allowing us to assign absent status to these OTUs in animals failing to meet the criteria for OTU presence.”

11) Line220: please define chimera

Done. Chimeras are artificial sequences generated from two or more distinct parent DNA templates during PCR amplification (Ashelford et al. 2006).

12) Line 228: mention Table 1 here.

Done. “Thus, only OTUs for which there were at least 500 reads across all animals in the dataset (for which present and absent statuses could be assigned), and where reasonable certainty of pathogenicity could be established from the literature (see Table 1), were considered in analyses of the pathogen community.”

13) Table 1: This table is very useful. However, it would be “perfect” if there were also the references of the articles showing, or at least suggesting, the pathogenicity of the different bacteria. Also, in the 3rd column header, change "Nuimber" to "Number".

Done. And thank you for catching that type-o!

14) Line 235: I am not totally convinced by "co-exposure" (but I have no better suggestion...). In my opinion, "co-exposure" is associated with a time notion that cannot be captured by the approach used here (co-exposure = simultaneous exposure?).

Totally agreed, but since there is currently no jargon for this, we instead are defining that time window as anytime in the past. I *could* make up some jargon, such as History of exPosure (HOP) – meaning we could say “co-HOP”. Or something more clever. But I’m not sure this is the venue for that. Maybe a short opinion piece is in order?

15) Line 251: I agree with Recommender's comment about using drop1. However, the authors seem to want to keep this method.

Please see our response to Recommender’s comment above.

16) Lines 370-371: It would be well to mention instead that R. norvegicus was not included in the first analysis (3.2.1)

Done.

17) Lines 452-458: I am not convinced that the two models here are really necessary. Given that R. norvegicus hosts had the second most diverse pathogen community and that only rats are present in farm habitats, it is quite obvious that farm habitats are among the habitats with the highest diversity.

We agree with the reviewer here that the two analyses are redundant, and have removed the habitat analysis from the manuscript, and results from Table S4.

18) Lines 466-475: More information is needed in the legend of figure S5. I can't make the connection between the result part and this figure.

Done. This also helped me catch a type-o on line 467 where *Mi. agrestis* should have been *Mi. arvalis*. So, much appreciated!

19) Figure S2A: please add the horizontal bar mentioned in the caption or modify the caption.

This was actually Figure S6A, but great eye!! Thank you for catching this. The horizontal bar has been put back in place.

20) Lines 530-642: I would like to congratulate the authors because despite the large number of tests performed (especially from part 3.3 onwards), the manuscript is really pleasant to read. The tables in the supInfo are really well structured and really help to understand. However, this requires a lot of back and forth between the MS and the SupInfo.

We appreciate the reviewer's comment, and the understanding that we took great care to try and balance between readability and providing all details within the main text.

However, we agree that it could be nice to provide some of the results within the main text, such as the MCA contribution figure. This provides visual support of actual results on the associations rather than asking readers to believe in the summary of our interpretations. It is now main Figure 4 instead of supplemental figure S9 (in the previous version). Hopefully this helps with having to flip back and forth.

21) Line 691: "Here, we found to two distinct…." removed "to"

Done.

22) Line 800: add a full stop (.) at the end of the sentence.

Done.

23) Line 889: perhaps authors could add a reference here (e.g. Malmqvist et al 2004, 10.1098/rsbl.2003.0120 or Gutiérrez-López et al 2020, 10.3389/fevo.2020.569230)

Done.

24) Lines 984-987: please see e.g. Galen et al 2019, 10.1111/1365-2656.13089

Ref added.

25) It might be appropriate to point out in the limitations of this study that the detection of bacteria was only done in one organ (it is very likely that the authors missed other bacterial pathogens, please see: Mangombi et al 2021 10.1371/journal.pone.0248244, Genné et al 2021, 10.1038/s41396-021-00939-5).

Fully agreed. We have now added a sentence in lines 915-917. "For instance, our decision to screen only the spleen means we could have missed evidence of exposure to pathogens that can only be found by screening the liver, kidney or brain (Mangombi et al., 2021)."