Date

Dear Dr. Gareth Jenkins,

We thank you, as well as the Associate Editor and the reviewers for these comments that without doubt have greatly improved our manuscript. Please find here our detailed response. We attached an annotated document with changes highlighted in yellow. Moreover, as suggested, we will move our supplementary material as an appendix. We hope that these improvements will make our manuscript acceptable for publication in E&E.

Best regards

Alice Balard for the authors

22-Jul-2020

Dear Dr. Balard:

Many thanks for your manuscript ECE-2020-06-00967 entitled "Coupling between tolerance and resistance differs between related <i>Eimeria</i> parasite species: implications for co-evolution with their mouse hosts" which you submitted to Ecology and Evolution.

As you will see below, comments from our Associate Editor and referees suggest a major revision before your paper can be published. Their comments should provide a clear road-map for you to revise, hopefully improving the clarity and rigour of the presentation of your work. I'd also encourage you to move your supplementary material into the main text (or as an appendix). It makes it much easier for readers to access this information, as they can download the entire paper as a single file. We have an Editorial explaining our thinking on this, if you're interested: https://onlinelibrary.wiley.com/doi/full/10.1002/ece3.2101

Once again, thank you for submitting your manuscript to Ecology and Evolution and we look forward to receiving your revisions.

Sincerely,

Dr. Gareth Jenkins

Editor in Chief, Ecology and Evolution

gjenkins@wiley.com

-- Instructions --

Before submitting your revisions:

1. Prepare a response to the reviewer comments appended below in point-by-point fashion. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response, using line numbers to indicate where changes were made. If your manuscript does not currently include line numbers, please add them during revision..

2. Prepare a revised manuscript (word document), highlighting the changes you’ve made. Save this new document on your computer as you will be asked to upload it during the revision submission process. NOTE: Please be sure to keep in mind reviewer comments and incorporate your responses within the manuscript. Here at Ecology and Evolution, we are looking for you not only to respond to the comments, but to integrate them into the MS. This will reduce the concerns that others in your field may have, as reflected in the referee comments. There may well be areas where you disagree; for example, you may want to write, "A reviewer suggests that... However, I disagree because...". In any case, please try to address all of the concerns that are raised within the manuscript.

3. In addition to your revised manuscript with changes highlighted, please also save a “clean” copy where the changes are not marked.

4. If you have not yet uploaded an organism photograph, please consider doing so (although this is optional) and, if appropriate, designate this photograph as Figure 1. This may require you to include one or two explanatory sentences at the beginning of your manuscript. TIFF format files with a resolution of at least 300 dpi are preferred. If the photograph was not taken by the authors, please credit the photographer in the figure legend; please also ensure the image is not under copyright which would prevent it being published in Ecology and Evolution. Please direct any questions about this to ecoevo@wiley.com.

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Log into https://mc.manuscriptcentral.com/ecologyandevolution and click on Author Center. Under author resources, use the button “Click here to submit a revision”. PLEASE DO NOT SUBMIT YOUR REVISIONS AS A NEW MANUSCRIPT.

2. Follow the on-screen instructions. First you will be asked to provide your “Response to Decision Letter”—this is the response to reviewer comments that you prepared earlier.

3. Click through the next few screens to verify that all previously provided information is correct.

4. File Upload: Delete any files that you will be replacing (this includes your old manuscript). Upload your new revised manuscript file with changes highlighted, a “clean” copy of your revised manuscript file, any replacement figures/tables, or any new files. Once this is complete, the list of files in the “My Files” section should ONLY contain the final versions of everything. REMEMBER: figures/tables should be in jpeg, tiff, or eps format.

5. Review and submit: please be sure to double-check everything carefully so that your manuscript can be processed as quickly as possible.

Deadlines:

Because we are trying to facilitate timely publication of manuscripts submitted to Ecology and Evolution,your revised manuscript should be uploaded as soon as possible. If it is not possible for you to submit your revision in 2 months, we may have to consider your paper as a new submission. If you feel that you will be unable to submit your revision within the time allowed please contact me to discuss the possibility of extending the revision time.

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Associate Editor Comments to Author:

Associate Editor

Comments to the Author:

This study investigates the association between resistance and tolerance of different Eimeria species in different strains of the mouse host to determine whether there is evidence of adaptation to the host. Two reviewers found the manuscript to be on an interesting topic and the study to be largely well-executed. One reviewer had more minor comments focused on clarifying aspects of the history of the host and parasite populations, and made suggestions for other aspects for the Discussion. Another reviewer was more critical, focusing largely on aspects of the analyses, and in particular, the lack of justification for certain decisions, including the sample sizes and the methods used to analyse the data. If the authors pay close attention to these comments, particularly strengthening the rationale for their statistical approaches, this will make a nice paper.

We thank the Associate Editor for his/her comments. We modified the manuscript and provide here a detailed answer to the reviewers concerns (see mainly comments XXX). We hope that our manuscript has improved and is now suitable for publication in E&E.

C1. I do have one minor comment to add myself. The association between resistance and tolerance depicted in figure 5E is supposedly strong, but it looks as though it’s driven entirely by one data point (number 8). The suggestion that this is unlikely to be a statistical artefact is itself based on a marginal result, and as such I’m not sure that the association in figure 5E is especially robust.

This is a very good comment. We tested the significance of this association by removing the point 8 (corresponding to one mouse strain, PWD) and found a similar results ; we did not write that information in the previous version and see now that it was missing. We added in Appendix a figure (figure x to do) with the removed outlier, and added in-text explanation (xxx).

Good comment, same analysis without the mouse strain 8, strong result. Change scale in figure? add figure in annex?

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author

This is a very interesting and well explained study that addresses an interesting and important topic. The paper looks at the coupling between tolerance and resistance in different parasite species. In addition it asks whether there is any evidence for adaptation to specific parasite clones in different mouse host strains. The introduction, methods and discussion are well written and explained, and the results are mostly well presented with a few omissions.

C2. My major concerns are about the sample sizes used and the presentation of the results. First, I’m a little concerned about the ethics here. The numbers of mice used for each treatment combination is very low. It is just as unethical to subject animals to treatments that impact their welfare in numbers that are too small, as too large. Too large impacts more animals than is necessary to answer the question, too small kills animals for no real purpose as the numbers are not large enough to detect an effect. This seems to be the case with the strain by genotype effects shown in figures 3A and B. There are apparent differences between the strains that would likely be detected if more than 6 or 7 animals were used for each treatment. Why so few? This seems like an incredibly small number to determine effects in this kind of study. I would expect to see that a power analysis for this study had been carried for the ethical approval, indicating how many animals were required to show differences between groups. If the number of animals that could be used was limited by time, space or money, then the ethical option is to ask fewer questions with the same number of animals.

We understand the concern of the reviewer as ethics in animal experiment is a crucial point that we considered. A preliminary study was conducted with NMRI mice and stronger effects were detected (unpublished data). In the present article, we wanted to test differences between mouse strains, so the statistical unit is the strain level. The mice used in the present article are wild-derived inbred strains and there is indeed a limitation in the number that can be used. For statistical power at the strain level. We have 8 strains, which is more than numerous papers. Maybe integrate a sentence ?

line 128: « We used eight mouse strains in total, as follows: four wild-derived inbred mouse strains from which we generated four groups of F1 hybrids »

For coupling/non-coupling, power comes from number of host strains. Hence ethically 3 groups with 20 mice each would be less powerful. -> Simulation to show this. 8 groups, more mice, around the same means, re-simulation 1000 times, how often we don’t find the same. The means might change. → do that

C3. Second, some of the results are oddly presented with no justification. In some cases data is logged, in others not. Main effects are not presented, data are presented in a way that doesn’t match the analyses etc. in one case the analysis presented does not test the stated hypothesis. I have detailed these queries below.

We thank the reviewer for this comment and corrected our manuscript accordingly, see answers to comments below for detailed corrections.

-> interaction effect between parasite species and mouse strains/species

show WHY we can separate

* posthoc tests

139 no hybrids

C4. A more minor comment is around the framing. There is some discussion of hybrid zones and the role of parasites. Hybrid mice are used but there is not analysis or discussion of whether hybrids are different to the parental genotypes, more like one than the other, what this might mean for hybrids in the field? This seems like an odd omission.

In this article, we used wild-derived inbred mice that are rather complex to obtain. Hybrids were used (1) to increase the number of strains, and therefore the statistical power, and (2) for representing a baseline for future work in which we want to investigate the difference of resistance and tolerance in hybrids compared to pure strains in a controlled environment. Such work will necessitate more mouse strains. As this is out of the scope of the present manuscript, we chose to not mention it. As it seems like an odd omission, we added the following :

line xx : « blabla »

C5. Lines 209-213: use log OPG, any evidence of curvature in the fits?

We used log OPG only for visualisation, but did not modelise at this level, always on the raw data. For clarity we homogenized and plotted now all on the raw scale (see new Figure 3A, 4A, 5A).

C6. Line 226: why? Surely if the interaction term is not significant then this is not statistically justified as it inflates the type 1 error rate?

We thank the reviewer for this comment, and replaced this step-wise approach that could indeed inflate type 1 error by post hoc tests with correction for multiple testing, a follow :

We changed « For each of our model, we also asked within each parasite isolate if the response differed between mouse groups using likelihood ratio tests (G) as described above. » (previously lines 226-227) for :

« For each of our model that showed a significant interaction term, we also asked within each parasite isolate if the response differed between mouse groups using likelihood ratio tests (G) as described above. In the case of a non-significant interaction term, we performed post-hoc tests corrected for multiple testing (Tukey’s test using the R package multcomp (REF)) to compare within all pairwise comparisons between groups (parasite isolate-mouse strain) »

multicomp::glht(model, linfct = mcp(parasite.mouse = "Tukey"))

We reorganised the text accordingly, adding the following :

« 3. Comparison of resistance-tolerance coupling between *E. ferrisi* and *E. falciformis »line 306*

C7. Lines 258-262: why not log OPG and use pearsons?

As a non-parametric test, Spearman’s rank correlation test is less sensitive to outliers than Pearson’s test, and also more stringent which is important for us to avoid false positive correlations. As asked by the Asssociate Editor, we tested also this correlation removing the point of mouse strain 8 (PWD) (see comment C1)

C8. Lines 294-296: what was the main effect of parasite isolate?

We chose to present only significant effects. We understand that this is confusing, and added now non-significant effects (see lines xxxx)

C9. Lines 302-305: I’m not sure you’ve specifically tested for this? To test whether strains are better (or even worse) adapted to their own parasite (the parasite might be winning this co-evolutionary battle) you would need to pool the domesticus and musculus and ask whether ‘matched’ i.e. eastern w/ eastern and western w/ western was different ‘unmatched’ i.e. EW and WE. I don’t think they will be, looking at the data, but you still need to specifically test for it.

-> WW + EE = matching vs WE + EW = non matching. Test the difference. Same model.

We have neg results, we want max power to exclude false negative so we do that

low power, pbatic for local adaptation, we use matching/non matching to increase number per group.

C10. Lines 334-336: this is confusing, you found a positive correlation between OPG and tolerance, which indicates a negative correlation between tolerance and resistance. However, again, this fit is terrible, try log OPG.Figure 3: What do the error bars represent? Is this raw data or predictions from the model? If the latter, for A and B, given no significant interaction, why plot the parasite strains separately? Why is OPG plotted on a log scale for A but not for C?

This is a good point raised by the reviewer. It was probably confusing: we did not fit a statistical model at this point, but used correlation, line is for illustration, we add that for clarity to the figure caption: line is for illustration. 95% CE from the model

log scale -> homogenize

Error bars figure 3 -> 95% CE from the model

C11. Figure 4: As for 3, why is OPG plotted on a log scale for A but not for C,D or E?

As mentionned in comment C5, log scale was only for visualisation, we plotted now everything on the raw scale to avoid confusion.

Reviewer: 2

Comments to the Author

This article reports the results of careful experiments on infection resistance and tolerance in pure and crossed wild-derived mouse strains and wild-derived Eimeria isolates. The experimental subjects are well linked to a natural context in terms of the Mus musculus x Mus mus hybrid zone in central Europe. The rationale for the study is clearly set out and compelling, and the experiments are clearly described and analyzed. The authors find a certain pattern of significant results and interpret these in a reasonable and interesting way, leading to the introduction of an interesting idea about whether correlation between tolerance and resistance affects the tendency of host-parasite systems to undergo antagonistic coevolution. Broadly I found the article clear and I feel usefully enlightened and glad that I have read it – I am sure many other readers will as well.

The data are what they are, and there may be some limitations in terms of the inference drawn from the pattern of results. The negative correlation between resistance and tolerance proxies in Eimeria falciformis infection is convincing up to a point, but there is also the slight concern about autocorrelation (although, to be fair, the authors are very explicit about this). C12. Also, there is a lack of a direct statistical test for a difference in the relationship between tolerance and resistance in the two Eimeria species infections.

-> non parametric test of difference with a factor non parametric glm kinda. Compare 4E and 5E. lm(res~tol

The authors depend on qualitatively comparing coupling or uncoupling based on the pattern of test results within the different compartments of the study – and this could be affected by statistical power issues within compartments.

C13. Some of the co-evolutionary discussions assume that the resistance and tolerance phenotypes are underpinned by genetic variation. But what if environment or genotype x environment effects – which could be confounded with strain or isolate - are important? Furthermore, it is likely that laboratory animals will often adopt phenotypes (including immunological and infection phenotypes) very unlike those in the wild. If they have been bred or passaged in the laboratory they may also have undergone unrepresentative genetic changes. For this reason, more details should given on the husbandry / passage history of the host strains and parasite isolates, although, in general, the form of the study has been very clearly represented.

add husbandry info

Notwithstanding, I think that, overall, this article is detailed and relevant work that will be interesting for many readers.

Specific points:

C14. 71 “Eventually, ….” This doesn’t seem to be the right word, implying a timeline – but the citation is an early one.

We removed « eventually »

C15. 50-52. But arguably the determinants go beyond metabolic costs and involve the disruption of function and ultimately fitness. These arguments, and those above, don’t seem to quite paint a full picture.

Xxx dvp argumentation

C16. 138 This is quite high variation in mouse age, given the life-span of the lab mouse.

This is indeed rather high, but mainyl driven by few individuals, and more importantly not clustered by mouses strain : indeed, for all mouse strains, the means are very close. We added this information line xxx :

«  Age of the mice at the time of infection ranged between 5.6 and 21.4 weeks, with a mean for each eight mouse strains ranging between 11.2 and 14.7 weeks. »

C17. 169. If these were pinworms (can anything be said about their identity, beyond just “nematodes”?), then I think that the authors are fully justified in just going ahead and assessing the Eimeria results regardless. Other types of nematode might be more of an issue, but pinworms are what is typically found in many laboratory colonies.

We identified classically found *Syphacia* sp. and *Aspiculuris* sp. eggs and added the information line 171 check : « (*Syphacia* sp. and *Aspiculuris* sp.) »

C18. 195-199 Although generally the acid test for what distribution should be used for model analyses rests on the assessment of model residuals, rather than lumped raw data.

Xxx read on that

C19. 217 “either”?

Thanks for spotting that, this is a typo, we removed it.

C20. 291-222. Consider refining the wording of this sentence.

We replaced the previous sentence « *For tolerance, we performed a linear regression with null intercept (as each mouse was controlled against itself at start of the experiment, before losing weight or shedding parasite), modelling relative weight loss as a response of maximum OPG interacting either mouse group, parasite isolate and their interaction*. » by

« xxx »

C21. 226. models

We corrected, thanks

C22. 272 with the

We corrected, thanks

C23. 298. “Eventually” doesn’t seem to be the right word.

Xxx see if we change statis

C24. 298-302. This is getting a bit confusing or is a lapse of explanation. The wording of this sentence implies a linear regression between mouse strain and parasite isolate (two factors), but this doesn’t seem make sense, or at least the meaning is not fully clear. Suggest re-wording.

We changed and rephrased, see comment of reviewer 1 (C9)

C25. 318 Eventually?

We replaced by « moreover »

C26. 363 literature

We added xxx.

C27. 365 tolerance mathematically

We corrected, thanks

C28. 366 meaningful

We corrected, thanks

C29. 367. Why stepwise? This implies one comes before the other – but this isn’t

necessarily so.

We corrected for « double »

C30. 368-370. Check spellings and wording of this sentence.

xxx

C31. 372-375. This needs to be explained better.

xxx

C32. Fig. 2 legend. Host relative weight? What hosts are these data for?

We added « host » and precision : « All mouse groups are pooled together for each parasite isolate. »

C33. Fig. S2.3 legend line 46 …in E. falciformis?

We corrected, thanks (TO DO)