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PROCEEDINGS B

Whose trait is it anyways?: Coevolution of joint phenotypes and genetic architecture in mutualisms

Anna M. O'Brien, Chandra Jack, Maren L. Friesen and Megan E. Frederickson

Article citation details
Proc. R. Soc. B **288**: 20202483.
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Review timeline

Original submission:	4 July 2020
1st revised submission:	15 October 2020
2nd revised submission:	7 December 2020
Final acceptance:	7 December 2020

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

Review History

RSPB-2020-1604.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Reject – article is scientifically unsound

Scientific importance: Is the manuscript an original and important contribution to its field?
Acceptable

General interest: Is the paper of sufficient general interest?
Poor

Quality of the paper: Is the overall quality of the paper suitable?
Poor

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
No

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
O'Brien et al. consider the evolution of ``joint-phenotypic traits'', traits determined by the loci in multiple partners of a mutualism. Grounded in diverse biological examples ranging from plant-pollinator, plant-rhizobia, and host-microbiome interactions, understanding the evolution of such joint phenotypes is fundamental for our understanding of these systems. Using a simulation approach the authors first simulate evolution of joint-phenotypes, examine the resulting dynamics of genetic variation of joint -phenotypes over the course of evolution, and finally perform in silico GWAS experiments on the resulting host and microbe genomes. Together these three components (simulations, analyses of genetic variance, GWAS) constitute a significant undertaking and I believe have the potential to contribute significantly to our understanding of coevolving traits in mutualisms.
I do, however, have four particular reservations about the present framing of the work, simulation design, and presentation. First, at present the work is not hypotheses driven. The questions posed are highly exploratory with unclear a priori description of the qualitative and quantitative patterns to be examined. Second, the work would benefit greatly from a deeper connection to existing theoretical literature on the coevolution of quantative traits. Third, there are a number of missing simulation details that are essential for the understanding of the results. Finally, I am skeptical of the mechanismal interpretation of the results in the top left panel of Figure 2.
1) At present the work is not question/hypothesis driven. The study aims (as laid out in Ln 54-59) are to ``ask how trait and allele evolutionary trajectories are determined'', ``asses the resulting patterns of genetic variation'' and ``validate methods to infer the basis and selective history of a multi-genomic trait''. It remains unclear throughout, however, exactly how these questions are to be addressed both qualitatively and quantitatively.
In the GWAS section for example, it is unclear whether the aim is to a) characterize the patterns of join-trait causative loci in general or to develop methods to distinguish between loci in ``conflict'' versus loci underlying aligned traits.
2) The work has not been adequately framed in the context of existing work on the coevolution of phenotypic traits in mutualisms. There has been substantial work on the evolution of quantitive traits in mutualisms (e.g. Nuismer 2017 Ch 3). Unfortunately, I am not super familiar with the mutualism literature so sorry for not provide more precise references. There are also several points where the work would benefit from expanding on the literature referenced (e.g. Ln 50 and with respect to fitness alignment and the connection to Heath et al. Ln 334).
3) There are a number of missing details in the simulation design. Most importantly how are the effect sizes (aH1, aH2, and aM1) drawn? How are they distributed, what are their means. This is relevant first wrt evolutionary dynamics where if (as I am currently assuming) they are all drawn

from the same distribution of phenotypic effects this will inherently favour evolution in the host as it has effectively twice as many loci. Similarly, are the loci biallelic? What is the resulting distribution of dominance coefficients?
4) As I understand it you have attributed the results in the top left panel to the pathogen constraints due to clonal interference, however I am unsure if this is robust. First and fore most the hosts have twice as many loci, as described above this gives them
inherently more potential (unless adequately corrected) as a result they also have twice the mutational target size and hence are lest limited by genetic variation when beginning from a monomorphic state. Finally, it is unclear how this result depends on the initial conditions. As shown, the host trait optima is further from the initial condition and hence the host is under stronger selection initially which once again could produce the observation seen. Lastly it is unclear how robust this result is to stochasticity, you show only a single example but it is unclear whether this same trajectory arises in multiple runs. To further test whether the dynamics are indeed the result of clonal interference I suggest examining the case without recombination in the host to distinguish factors due to diploidy vs. asexual reproduction.
Some Specific Comments:
Ln 4-5 & ln 32-33: Why would we expect joint traits to be more common in mutualisms? With respect to this I am confused about the holobiont example in Ln 37 and how this pertains to why mutualisms should in general have more joint traits or joint traits under stronger selection. My intuition would be that the strength and prevalence of joint traits would depend more on whether the interaction was between specialists or generalists than an antagonism vs. mutualisms.
Ln 10: What does ``fitness feedbacks'' mean?
Ln 21 vs. Ln 28: I think there is a difference between an individual carrying a trait that has fitness consequences on another species (e.g. host immunity) and a join trait as you discuss here.
Ln 48-50: Expand on this previous work.
Ln 64-66 "thus, for example...". Revise grammatical mistake?
Ln 199: What is the value of L?
Equn 1: How are a's drawn? What are the maximum phenotypes? How do these compare to the optimal phenotypic values?
Equn 4: It would be useful to have plot the net fitness landscape. It is unclear how much epistasis there is given the multiple factors influencing fitness.
Ln 240: The effect of recombination also depends on the shape and form of epistasis. Given that there is substantial epistasis for fitness in this model this may be extraordinarily important.
Ln 264: "equal links to fitness" Expand on this. This is non-trivial to both define and prove.

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The manuscript by O'Brien and colleagues models the evolution of a phenotypic trait that is underlain by genetic variation in two interacting organisms: a microbe and its host, which may have the same or different optimum values of the trait. Key findings include that positive fitness feedbacks can partially resolve conflicts between the partners over the optimum trait value, so that both partners "compromise" on their evolved breeding values. Alternatively, without a fitness feedback, both partners may evolve to "overshoot" their optimum trait values as a means of compensating for the partner's opposing effect; as a result, removal of one partner for the system could still result in maladaptation of the other. The manuscript also demonstrates that standard genome-wide association study methods (GWAS) can be used to detect the genetic basis of a trait across both partners' genomes.

This work is novel, thought-provoking, clearly written and presented, and will make a valuable addition to the literature on the evolution of host-microbe interactions. This paper presents a novel framework for understanding the forces underlying the evolution of mutualisms in general, while bypassing the messy problem of whether host-microbe associations are heritable. I think that this framework will inspire other researchers to take new approaches to studying host-microbiome evolution and will lead to advances in the field. In my opinion, it brings some new clarity to how we should be thinking about mutualistic interactions and the problematic concept of "cheating". The rationale for this work is well presented with a substantial introduction section and literature review. Most of the motivating examples are from plants, but the framework is applicable to any host-microbe pairing.

My questions and suggestions for improvement are mostly minor.

One variant of the model investigates a scenario in which the trait is selectively neutral in the microbial partner (Fig. 2, left column). To what extent would the outcome differ if it were the host in which the trait was neutral?
In general the authors do a good job of pointing out the simplifications they made for their model and how those simplifications may affect the results (e.g., discussion of differing mutation rates, lines 226-230, generation times and dominance, line 240). One really key issue was not discussed, however: the reality that many or most host-associated microbes can survive and reproduce perfectly well without a host (e.g., free-living in soil or water). There have been some good papers

recently showing that selection pressures on rhizobia differ between free-living and host-associated environments (e.g. https://doi.org/10.1111/evo.13807). Modeling the effects of this complication may be beyond the scope of this paper, however, it is an important issue that should at least be mentioned. A more general formulation of this issue is the idea of "alternative hosts" (or for the host, alternative symbionts) that will shape the evolutionary trajectory of the focal trait. In reality the fitness feedbacks between partners are probably not as symmetric as modeled here.

Line 82: I think it is premature to claim that microbes influence "most" plant functional traits. "Many" is sufficient to get the point across here.

Lines 278-283: This is an excellent insight on the odd behavior often observed in eukaryotes forced to develop sans microbiota.

Figure 2 - it is difficult to resolve the colors of the horizontal dashed lines even when zoomed in. Perhaps supplement with colored block arrows along the vertical axis to make it clearer?

Decision letter (RSPB-2020-1604.R0)

25-Aug-2020

Dear Dr O'Brien:

I am writing to inform you that your manuscript RSPB-2020-1604 entitled "Whose trait is it anyways?: Coevolution of joint phenotypes and genetic architecture in mutualisms" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.
4) Data - please see our policies on data sharing to ensure that you are complying (https://royalsociety.org/journals/authors/author-guidelines/#data).

To upload a resubmitted manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with

Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Professor Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This manuscript investigates the emerging topic of understanding the genetic architecture of traits influenced by multiple genomes, with a particular focus on mutualisms. It is an exceptionally well-written and accessible piece of work, giving detailed background with many relevant examples, and clear explanation and interpretation of the simulations. The manuscript was evaluated by two reviewers: Reviewer 2 was largely positive about the submission, whereas Reviewer 1 had a number of concerns that should be addressed before recommending publication. My additional comments:

One aspect missing from the background was that more context for the study is required. What recent work has investigated quantitative genetic models of mutualisms/multiple genomes? What have been the most recent advances in the field and how does the current paper build on them? Has there been similar simulation and/or empirical work in this field?

There are some issues with understanding and interpreting the simulation study – please check that the analysis is reproducible and that all relevant terms are defined. Like Reviewer 1, I would have appreciated more explanation of "fitness feedbacks".

Susan Johnston.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
O'Brien et al. consider the evolution of ``joint-phenotypic traits'', traits determined by the loci in multiple partners of a mutualism. Grounded in diverse biological examples ranging from plant-pollinator, plant-rhizobia, and host-microbiome interactions, understanding the evolution of such joint phenotypes is fundamental for our understanding of these systems. Using a simulation approach the authors first simulate evolution of joint-phenotypes, examine the resulting dynamics of genetic variation of joint -phenotypes over the course of evolution, and finally perform in silico GWAS experiments on the resulting host and microbe genomes. Together these three components (simulations, analyses of genetic variance, GWAS) constitute a significant undertaking and I believe have the potential to contribute significantly to our understanding of coevolving traits in mutualisms.
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of compensating for the partner's opposing effect; as a result, removal of one partner for the system could still result in maladaptation of the other. The manuscript also demonstrates that standard genome-wide association study methods (GWAS) can be used to detect the genetic basis of a trait across both partners' genomes.

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Lines 278-283: This is an excellent insight on the odd behavior often observed in eukaryotes forced to develop sans microbiota.

Figure 2 - it is difficult to resolve the colors of the horizontal dashed lines even when zoomed in. Perhaps supplement with colored block arrows along the vertical axis to make it clearer?

Author's Response to Decision Letter for (RSPB-2020-1604.R0)

See Appendix A.

RSPB-2020-2483.R0

Review form: Reviewer 2

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors have done an admirable job of responding to the issues raised by both reviewers and the editor. I particularly appreciate the references added to the revised version that further clarify this paper's relationship to past work. It reinforces my opinion that this will be a very useful addition to the literature. I have no further requests for edits.

Review form: Reviewer 3

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
In this paper the authors develop a novel simulation model to explore the (co)evolution of host-microbe joint-phenotypes and develop a GWAS approach to identify causal loci in multiple genomes. Overall I found the paper interesting and well written. From what I understand, the concerns of the previous reviewers have been addressed, making for a very nice paper that appears ready for publication. My only comments are on a potentially useful connection with multivariate quantitative genetics and to provide some minor suggestions for notation/terminology to help connect the model formulation with classical quantitative genetic theory. Please see the attached file.

Decision letter (RSPB-2020-2483.R0)

24-Nov-2020

Dear Dr O'Brien

I am pleased to inform you that your manuscript RSPB-2020-2483 entitled "Whose trait is it anyways?: Coevolution of joint phenotypes and genetic architecture in mutualisms" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into https://mc.manuscriptcentral.com/prsb 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. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

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

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository (https://royalsociety.org/journals/authors/author-guidelines/#data).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors

should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
NB. From April 1 2013, peer reviewed articles based on research funded wholly or partly by RCUK must include, if applicable, a statement on how the underlying research materials – such as data, samples or models – can be accessed. This statement should be included in the data accessibility section.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

6) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

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

Sincerely,
Professor Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member
Comments to Author:
Thank you for your contribution to this special issue on Wild Quantitative Genomics. Two reviewers and I have evaluated this revised manuscript. Overall, we are positive and enthusiastic about this piece of work – we believe it to be of excellent scientific importance and interest.

There are still some minor points to be addressed:

Reviewer 3 makes some suggestions on how the work could be better connected with terminology in multivariate quantitative genetics. This may be worth considering, or at the very least may merit some brief discussion.

There are lingering issues with reproducibility. Please signpost the supplementary methods and results more clearly in the main text – point out early on in the simulated case study where the full methods can be found. In what software was the simulation implemented? Reviewer 3 also pointed out some variables that were not clearly defined.

I suggest revising the language in lines 98-101 – on my first read, it implied that GWAS explains a small proportion of heritable variation and that inherited microbes explains the rest (or at least a large proportion).

Reviewer(s)' Comments to Author:
Referee: 3

Comments to the Author(s).
In this paper the authors develop a novel simulation model to explore the (co)evolution of host-microbe joint-phenotypes and develop a GWAS approach to identify causal loci in multiple genomes. Overall I found the paper interesting and well written. From what I understand, the concerns of the previous reviewers have been addressed, making for a very nice paper that appears ready for publication. My only comments are on a potentially useful connection with multivariate quantitative genetics and to provide some minor suggestions for notation/terminology to help connect the model formulation with classical quantitative genetic theory. Please see the attached file.

Referee: 2

Comments to the Author(s).
The authors have done an admirable job of responding to the issues raised by both reviewers and the editor. I particularly appreciate the references added to the revised version that further clarify this paper's relationship to past work. It reinforces my opinion that this will be a very useful addition to the literature. I have no further requests for edits.

Author's Response to Decision Letter for (RSPB-2020-2483.R0)

See Appendix B.

Decision letter (RSPB-2020-2483.R1)

07-Dec-2020

Dear Dr O'Brien

I am pleased to inform you that your manuscript entitled "Whose trait is it anyways?: Coevolution of joint phenotypes and genetic architecture in mutualisms" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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Paper charges
An e-mail request for payment of any related charges will be sent out shortly. The preferred payment method is by credit card; however, other payment options are available.

Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

You are allowed to post any version of your manuscript on a personal website, repository or preprint server. However, the work remains under media embargo and you should not discuss it with the press until the date of publication. Please visit https://royalsociety.org/journals/ethics-policies/media-embargo for more information.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Dear Prof. Susan Johnston and Reviewers,

We appreciate the efforts of the Reviewers and Prof. Susan Johnston as associate editor in reading and considering our manuscript. We appreciate especially that the strengths of our manuscript were noted, and that we received well-considered suggestions for improvements. We have now responded to each of these, and made a number of changes to our manuscript, including new supplemental analyses and figures. We respond to each comment in turn, but we address a few here, as reviewers and the editor highlighted similar suggestions.

Both reviewers and Prof. Johnston noted that some simulation and language details were not clear. We have added details to the main text, and pointed to our supplemental figures and text at more places in the manuscript, to better effectively highlight the material. We have also expanded our supplemental material.

Reviewer 1 and Prof. Johnston noted that more context in the previous literature would be useful. We agreed, especially as pertains to developing and introducing the simulation model. We have added new references (and moved others) in order to introduce our model in the context of previous simulation and theoretical work on either joint traits in other kinds of interactions or quantitative trait coevolution in mutualisms. We note we cannot find previous work on both, which was part of the original motivation for our manuscript.

Both reviewers pointed out several places where we could extend the simulation. However in the interest of not greatly adding to the length and scope of the manuscript we have included new simulation results only where possible to take on the task in a concise manner. For the other suggestions (role of differing genomic and reproduction traits including recombination, complex multispecies communities and temporally varying selection for microbes that may be free-living) we believe these are fruitful avenues of further research.

We think that our responses to these suggestions have substantially improved the quality of our manuscript, and we expect it to now be appropriate for publication in Proceedings B.

Sincerely,
The authors

Our responses are in green, below. Comments from the editor & reviewers are in normal black font color. Line numbers in the response refer to the **track changes version,** to facilitate locating relevant changes.

Associate Editor
Board Member: 1
Comments to Author:

This manuscript investigates the emerging topic of understanding the genetic architecture of traits influenced by multiple genomes, with a particular focus on mutualisms. It is an exceptionally well-written and accessible piece of work, giving detailed background with many relevant examples, and clear explanation and interpretation of the simulations. The manuscript was evaluated by two reviewers: Reviewer 2 was largely positive about the submission, whereas Reviewer 1 had a number of concerns that should be addressed before recommending publication. My additional comments:
Thank you. We appreciate the time you put into considering our manuscript, and your insightful comments. We further appreciate that you highlighted many of the strengths of the manuscript that we worked hard on.

One aspect missing from the background was that more context for the study is required. What recent work has investigated quantitative genetic models of mutualisms/multiple genomes? What have been the most recent advances in the field and how does the current paper build on them? Has there been similar simulation and/or empirical work in this field?
R1 also pointed out that links to previous modelling work were not sufficiently well described. We have clarified and expanded links between our work and recent work on coevolution of quantitative traits in mutualisms (matching trait models), and also with previous models for quantitative joint-phenotypes (though little to no work involves mutualisms). Importantly, we do most of this where we introduce the model, allowing comparison between the mathematical frameworks. Unfortunately, there isn't a lot of other previous work to reference, but we feel that these changes provide substantial improvements. See also our response to R1, below, for more details.

There are some issues with understanding and interpreting the simulation study – please check that the analysis is reproducible and that all relevant terms are defined. Like Reviewer 1, I would have appreciated more explanation of "fitness feedbacks".
To improve the clarity and interpretation of the simulation study, we have increased our attention to detail in defining relevant terms, and added more supplementary figures. Please see our detailed responses to individual comments below. We have also provided more background on fitness feedbacks, referencing examples from the mutualism literature as appropriate (see Lines 172-197) where we altered most of the paragraph explaining fitness feedbacks, and Lines 47-50 in the second paragraph, to introduce the concept much earlier.

***Note that line numbers in our response here and throughout refer to the track changes pdf, so that reviewers and editors can quickly compare.**

Susan Johnston.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

O'Brien et al. consider the evolution of ``joint-phenotypic traits'', traits determined by the loci in multiple partners of a mutualism. Grounded in diverse biological examples ranging from plant-pollinator, plant-rhizobia, and host-microbiome interactions, understanding the evolution of such joint phenotypes is fundamental for our understanding of these systems. Using a simulation approach the authors first simulate evolution of joint-phenotypes, examine the resulting dynamics of genetic variation of joint -phenotypes over the course of evolution, and finally perform in silico GWAS experiments on the resulting host and microbe genomes. Together these three components (simulations, analyses of genetic variance, GWAS) constitute a significant undertaking and I believe have the potential to contribute significantly to our understanding of coevolving traits in mutualisms.

Thank you R1, we agree it is a significant undertaking, and that it has the potential to contribute significantly to understanding coevolving traits in mutualisms. We appreciate your constructive critique of our manuscript. In particular we are glad that you pointed out a number of places where details were not clear, and that you (along with the editor) recommended more placement of our work in the context of past work. Your comments offered us the opportunity to greatly improve the manuscript.

I do, however, have four particular reservations about the present framing of the work, simulation design, and presentation. First, at present the work is not hypotheses driven. The questions posed are highly exploratory with unclear a priori description of the qualitative and quantitative patterns to be examined. Second, the work would benefit greatly from a deeper connection to existing theoretical literature on the coevolution of quantative traits. Third, there are a number of missing simulation details that are essential for the understanding of the results. Finally, I am skeptical of the mechanismal interpretation of the results in the top left panel of Figure 2.

1) At present the work is not question/hypothesis driven. The study aims (as laid out in Ln 54-59) are to ``ask how trait and allele evolutionary trajectories are determined'', ``asses the resulting patterns of genetic variation'' and ``validate methods to infer the basis and selective history of a multi-genomic trait''. It remains unclear throughout, however, exactly how these questions are to be addressed both qualitatively and quantitatively.

In the GWAS section for example, it is unclear whether the aim is to a) characterize the patterns of join-trait causative loci in general or to develop methods to distinguish between loci in ``conflict'' versus loci underlying aligned traits.

It is true that much of our paper is exploratory. Because this is such an open area of research (see responses to point 2), there aren't a lot of hypotheses out there to test, at least not for mutualisms specifically. Therefore, initial work is by necessity exploratory. However, we note that building a model-based simulation in and of itself is a hypothesis, and a hypothesis clarifying activity; i.e. by including fitness feedbacks, we hypothesize that the strength of these will matter for evolutionary dynamics.

Otto, Sarah P., and Alirio Rosales. "Theory in service of narratives in evolution and ecology." *The American Naturalist* 195.2 (2020): 290-299.

With respect to the GWAS section in particular, however, we agree that our statement of aims at the start of the section was not as clear as in other parts of the manuscript, and not as clear as it

should have been. We therefore altered the first paragraph of this section accordingly (lines 474-483)

2) The work has not been adequately framed in the context of existing work on the coevolution of phenotypic traits in mutualisms. There has been substantial work on the evolution of quantitive traits in mutualisms (e.g. Nuismer 2017 Ch 3). Unfortunately, I am not super familiar with the mutualism literature so sorry for not provide more precise references.

We took to heart the comment that we should make more explicit connections between our work and other work on the coevolution of phenotypic traits in mutualisms. That said, there are no other studies that we know of that explore the evolution of *joint* traits in mutualisms. Previous work has explored coevolutionary models for joint traits in antagonistic interactions, or for independent traits in mutualisms, e.g., trait-matching models. Nonetheless, we fully agree with R1 and the Associate Editor that more context would benefit the work. We added a new reference on joint traits in antagonistic interactions near the development of equations 2 and 3 (line 252, and also on lines 44-46, and retained general references to models of indirect genetic effects, lines 79, 270). We also expand our links to models of quantitative trait coevolution in mutualisms -- those that model 2 quantitative traits as a control on the outcome or presence of interactions (trait matching/difference equations). Chapter 3 (and the Nuismer 2017 book in general) is a useful reference as it efficiently covers many topics. Our thinking about how coevolution in mutualisms proceeds has been influenced by this stream of literature, and we thank R1 for reminding us of it. We added this reference and another along with discussion of the similarity and differences in approaches near equations 2 and 3 (at lines 258-270), as well as adding at other places where we discuss co-evolutionary models (lines 347-349, 581).

There are also several points where the work would benefit from expanding on the literature referenced (e.g. Ln 50 and with respect to fitness alignment and the connection to Heath et al. Ln 334).

We expanded on the sentences you flagged to clarify our meaning and cite additional references (lines 61-65, and lines 421-425).

3) There are a number of missing details in the simulation design. Most importantly how are the effect sizes (aH1, aH2, and aM1) drawn? How are they distributed, what are their means. This is relevant first wrt evolutionary dynamics where if (as I am currently assuming) they are all drawn from the same distribution of phenotypic effects this will inherently favour evolution in the host as it has effectively twice as many loci. Similarly, are the loci biallelic? What is the resulting distribution of dominance coefficients?

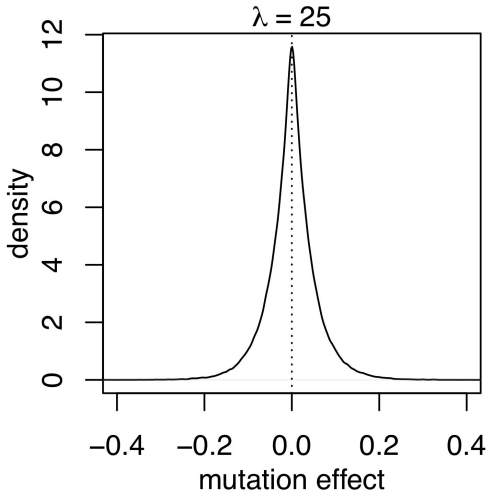
We thank the review for pointing out that simulation details were not clear. These details were outlined previously in the main text and/or supplementary materials, however we think perhaps the references to supplementary material in the main text were insufficient, and meaning was implied rather than explicit. We have made changes to clarify.

Specifically, vectors a_H and a_M are initially all 0. As the simulation proceeds, mutations may occur at an allele in an individual, and if so, are added to whatever the previous value for that allele was. We draw mutation sizes from an exponential distribution described by a rate parameter (lambda), but we then multiply that drawn number by a second drawn number (-1 or 1, drawn with probability 0.5). The resulting distribution has a mean of 0. We had explored the influence of various values for lambda in Figure S6. The distribution was described in the main

text, but we have now also added an example of the allelic effect size distribution as Figure S1 (copied below), mentioned at lines 288-292, in a sentence edited to improve description of this process.

Below R1 notes that a better presentation of the distribution is relevant for understanding the mutation effect sizes relative to the optimal phenotype values. Our new Figure S1 allows readers to intuit this. We don't provide details in the manuscript, as they change across different lambda and Z_{opt} parameters. However, for the reviewer, we offer some breakdowns in case it is of interest. Mutations are most commonly (about 40%) less than 1 percent of the distance between 0 and the closest optimal phenotype (where $Z_{optM} = 2$, some scenarios). Less than 1% of mutations arising from this distribution are equivalent to 10% or more of the same distance (>=0.2).

As the reviewer intuits, mutations for the host and the microbe are drawn from the same distribution, this should now be clear at line 290. Because mutations could theoretically add any number to an allelic effect, and because the only limit to the number of loci is N*ploidy and removal due to drift or selection (clarified at lines 291-292), this is best considered an infinite alleles model -- yet, because in practice, loci generally remain well below infinitely-allelic, we do not describe it as such. Further, we discretize alleles at each locus into linked biallelic states (as many biallelic states as necessary to cover all alleles) in order to run a GWAS. Discretization with a reasonable number of multiallelic alleles is equivalent to viewing each allele as a fully linked haplotype (no recombination within), i.e. a vector of states at biallelic loci. We improved wording at lines 482-489, and more clearly point readers to the supplement which has thorough explanation. We did not model dominance. Previously we had implied additivity within loci with the word "sum". We agree this is insufficient, and now specify "additive" and "no dominance" at this first introduction (line 246).



4) As I understand it you have attributed the results in the top left panel to the pathogen constraints due to clonal interference, however I am unsure if this is robust. First and fore most the hosts have twice as many loci, as described above this gives them

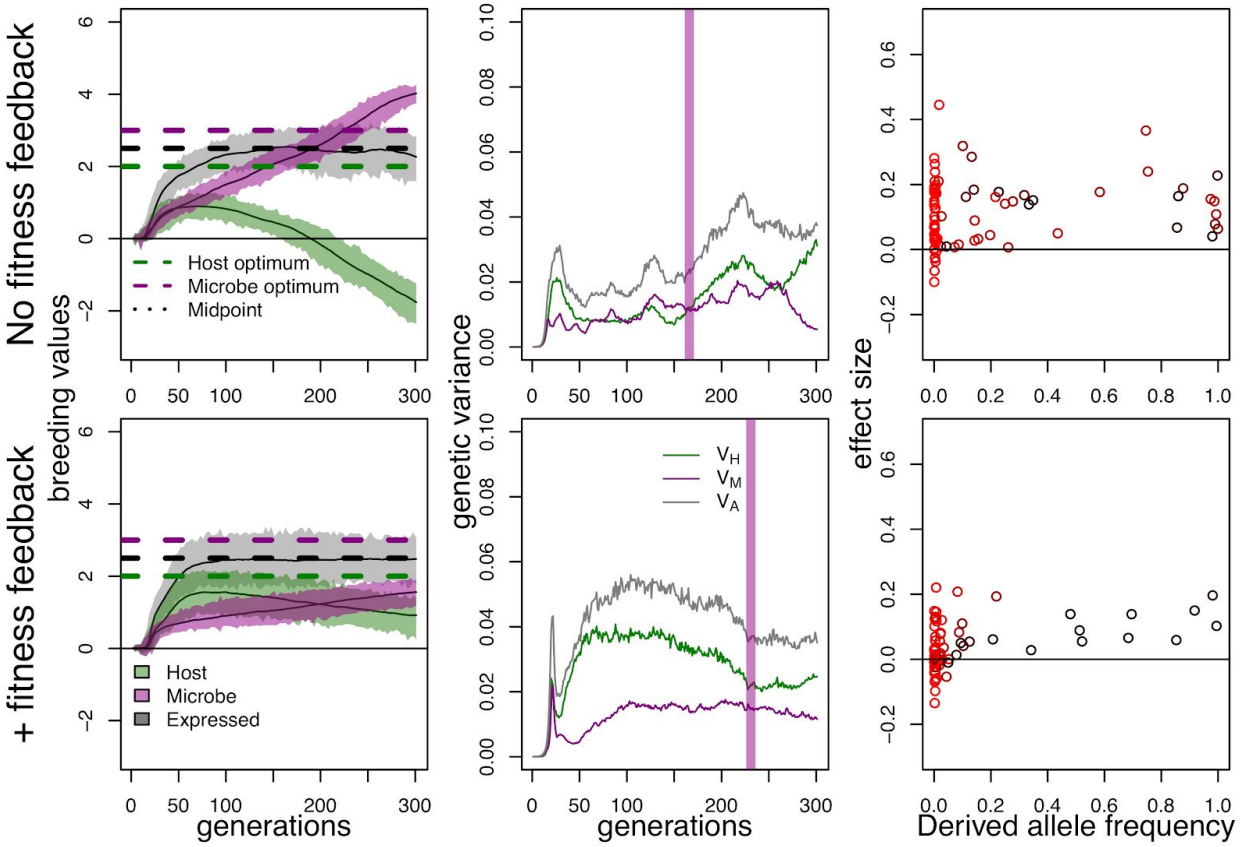
inherently more potential (unless adequately corrected) as a result they also have twice the mutational target size and hence are lest limited by genetic variation when beginning from a monomorphic state.

The number of loci were in fact different between hosts and microbes in most simulations to modify mutational target size to be the same -- microbes had twice as many loci as hosts. Hosts are diploid and so have two copies at each locus (we think the reviewer's meaning was to point this out). Thus, doubling microbe loci equalizes the number of copies of total loci across genomes, but not within loci. As mutation rate is equivalent across hosts and microbes, this means an equivalent rate of mutational inputs. This was previously mentioned, but we have now enhanced the clarity (lines 248-250).

Finally, it is unclear how this result depends on the initial conditions. As shown, the host trait optima is further from the initial condition and hence the host is under stronger selection initially which once again could produce the observation seen.

Based on this comment, we realized that we did a poor job referencing some of the work we have already done to test for dependence on initial conditions. We had already varied the extent to which the host optima was further, and the distance between the optima. As the reviewer correctly intuits this does alter the "final state" of the simulations, both alone, and when interacting with the strength of direct selection (omega) and indirect feedbacks (1-alpha, see Figures S6-S8). We now reference this more explicitly on lines 323-327 (end of "Simulation details")

Further, both R1 and R2 asked about reverse cases, R1 here for the case in which optima differ, and R2 for the case in which there was one optimum (see below). We now present flipped simulation examples of these in the supplement, combining the outcome summaries presented in Figures 1-3 for the other scenarios. In these cases outcomes for microbes now are similar to the outcomes for hosts in "unflipped" examples, with the exception that they still segregate less genetic variation than hosts (scenarios 1,4,6), or less than hosts do when they were under the same conditions (scenario 3). See new figures S2 and S3, where the figure for this scenario is S3. S3 & S2 are referenced at line 325 (end of "Simulation details", also see response to R2). S3 is also referenced at lines 341,387, & reproduced here:



Lastly it is unclear how robust this result is to stochasticity, you show only a single example but it is unclear whether this same trajectory arises in multiple runs.

While we do not present multiple runs of our scenarios in the main text, our conclusions are robust to multiple runs, and our examples are representative. We did investigate stochasticity across 5 runs for each combination of parameters for our sensitivity analysis (where we reported mean and variance across for various summary measures for the final state in S6 and S7). Except for the case of no direct selection on microbes, the parameters in main text examples fall within the range of the parameters replicated in the supplementary simulations. Further, we manipulated parameters affecting the nature of selection in combination (also replicated across 5 runs, summarized in S8). While these methods and results of this were previously present in the supplement, they were not adequately referenced in the main text. Previous references at (391,401,452,536) have been maintained. This content is now highlighted and clarified at first reference (lines 323-327). In the interest of minimizing additional figures, analyses and manuscript text, we have not added additional example scenario figures. We do, however, make all our code available if anyone wants to run them, either during review or post-publication.

To further test whether the dynamics are indeed the result of clonal interference I suggest examining the case without recombination in the host to distinguish factors due to diploidy vs. asexual reproduction.

This is a fair point. We currently don't fully disentangle the factors at play, and so we cannot be fully certain how much each contributes. Thanks for pointing this out. We have softened some of this language to indicate that various factors may play a role (301-309). We haven't performed this additional analysis (while a neat idea), because it's not a take-home message of ours to point out differences in host and microbe evolution (many others have pointed these out before), and because we don't expect these differences to affect the take-home messages we do emphasize (see results for flipping host and microbe parameters for the example scenarios, S2 & S3, explained above and below). This would be fascinating to explore in future studies! Especially because not all microbes are haploid, e.g. multinucleate fungi, which we point out on line 568.

Some Specific Comments:

Ln 4-5 & ln 32-33: Why would we expect joint traits to be more common in mutualisms? With respect to this I am confused about the holobiont example in Ln 37 and how this pertains to why mutualisms should in general have more joint traits or joint traits under stronger selection. My intuition would be that the strength and prevalence of joint traits would depend more on whether the interaction was between specialists or generalists than an antagonism vs. mutualisms.

We have re-written the paragraph containing previous lines 32-33 & 37 (paragraph on lines 35-53 in the track changes version) to better explain our thinking and to remove the holobiont example. Instead, we point out that symbiotic mutualism involves more close physical association in time/space (even on or inside partner tissue). In an antagonism the suite of traits involved might be more narrow because the interaction is largely about escape. In an obligate mutualism, you always interact with your partner -- in a pathogen/predator interaction some individuals escape.

Ln 10: What does ``fitness feedbacks'' mean?

Prof. Susan Johnston shared this concern. Please also see our response to her, above. We have now introduced fitness feedbacks earlier, in the second paragraph (lines 47-50), and expanded the paragraph fully explaining the concept on 172-197.

Ln 21 vs. Ln 28: I think there is a difference between an individual carrying a trait that has fitness consequences on another species (e.g. host immunity) and a join trait as you discuss here.We are not the first to point out the similarity between these two cases, and that indeed the difference may somewhat depend on the reader's view of what a phenotype is and whether fitness should be considered a phenotype. Queller (2014, now cited nearby, line 46) treats joint phenotypes in a similar fashion, and goes so far as to suggest that fitnesses of interactors may be considered joint phenotypes -- he formulates how a population of cheetahs may have heritable variation for gazelle fitness as some individuals may have alleles that increase their likelihood of predating any individual gazelle. We hope this clarifies our definition of joint traits.

Ln 48-50: Expand on this previous work.

We added a sentence here, at lines 62-65.

Ln 64-66 "thus, for example...". Revise grammatical mistake? Thanks, R1. We agree that this was awkward language. We have deleted it.

Ln 199: What is the value of L? On re-read, we agree this was somewhat unclear, thanks for pointing it out. We added a few words (lines 245-251), and reported as L_M and L_H, which is more

consistent with the different numbers of loci in host and microbes genomes in most simulations (usually double in microbes, but see supplementary analyses). L_M and L_H indicate the number of loci in microbe and host genomes respectively.

Equn 1: How are a's drawn? What are the maximum phenotypes? How do these compare to the optimal phenotypic values?

We agree that it is important the reader be able to find answers to these questions. We expect our response to point 3, above, both directly answers these questions (a's not drawn, rather sums of mutations which are drawn from an exponential distribution multiplied by -1 with probability 0.5; therefore there is no imposed maximum phenotype) and that the changes to the manuscript described there provide clarity. Our new S1, should allow the reader to compare effect size distribution to phenotypic optima for a typical value of the exponential rate parameter.

Equn 4: It would be useful to have plot the net fitness landscape. It is unclear how much epistasis there is given the multiple factors influencing fitness.

The net fitness landscape changes depending on the distribution of phenotypes in both populations. We had initially included a supplemental figure on this, but we did not explain that it depicted changes to the fitness landscape through time, nor did we reference it near Equation 4. We agree with the reviewer that this would be useful, and we have added the following text after Eq. 4 (lines 280-283):

"Because the impact of each fitness link depends on the distance to the optima of all hosts and microbes in the populations (Eq. 3), the fitness landscape and effects of fitness feedbacks can change through time (see Figures S4&S5)."

Ln 240: The effect of recombination also depends on the shape and form of epistasis. Given that there is substantial epistasis for fitness in this model this may be extraordinarily important.

The reviewer is correct in that there is both epistasis (GxG between host and microbe loci), and that recombination may alter effects of epistasis (as others before us have demonstrated, of particular relevance we note Arnold et al 2018). Like the reviewer's previous comment on recombination, we agree this would be fascinating to explore in further work, but would require a significant expansion beyond the current manuscript scope, so we do not include here.

Arnold, Brian J., et al. "Weak epistasis may drive adaptation in recombining bacteria." Genetics 208.3 (2018): 1247-1260.

Ln 264: "equal links to fitness" Expand on this. This is non-trivial to both define and prove.

We agree, in fact. Thanks, R1 for pointing out imprecise language here. What we meant was that the omega parameters were the same. Our original Figure S1 (now S4, which was the figure we had meant to reference here, not the original S2) shows how even with equal omegas ($\omega_M=\omega_H$), the position of the average trait value and the presence of a fitness feedback does continue to affect the strength of selection (steepness of curve) on hosts and microbes, so equal omegas does not indicate equal strength of selection. This now reads "equal direct trait-fitness links ($\omega_M=\omega_H$) in scenario 2" (line 336)

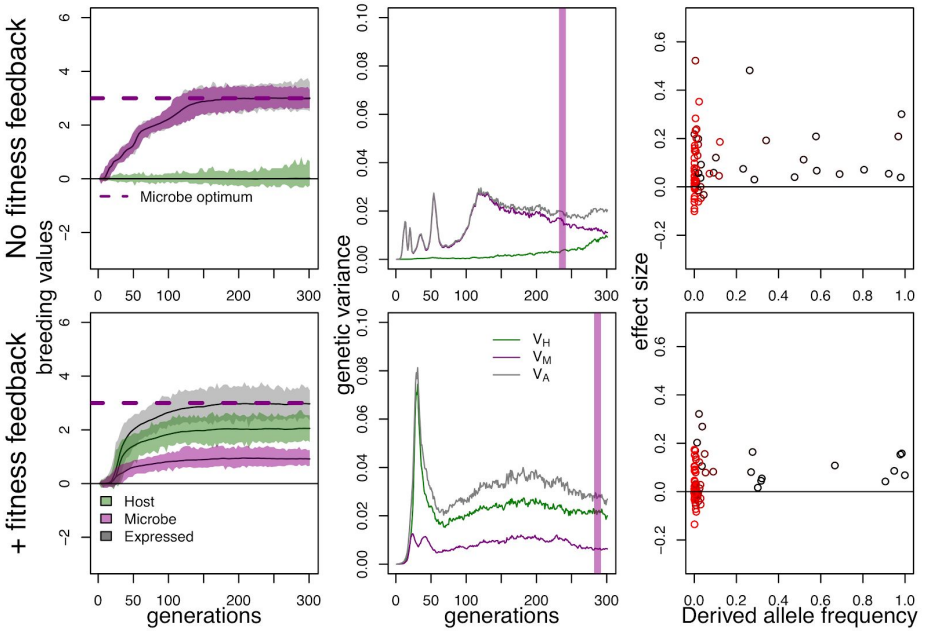
Referee: 2

Comments to the Author(s)

The manuscript by O'Brien and colleagues models the evolution of a phenotypic trait that is underlain by genetic variation in two interacting organisms: a microbe and its host, which may have the same or different optimum values of the trait. Key findings include that positive fitness feedbacks can partially resolve conflicts between the partners over the optimum trait value, so that both partners "compromise" on their evolved breeding values. Alternatively, without a fitness feedback, both partners may evolve to "overshoot" their optimum trait values as a means of compensating for the partner's opposing effect; as a result, removal of one partner for the system could still result in maladaptation of the other. The manuscript also demonstrates that standard genome-wide association study methods (GWAS) can be used to detect the genetic basis of a trait across both partners' genomes.

This work is novel, thought-provoking, clearly written and presented, and will make a valuable addition to the literature on the evolution of host-microbe interactions. This paper presents a novel framework for understanding the forces underlying the evolution of mutualisms in general, while bypassing the messy problem of whether host-microbe associations are heritable. I think that this framework will inspire other researchers to take new approaches to studying host-microbiome evolution and will lead to advances in the field. In my opinion, it brings some new clarity to how we should be thinking about mutualistic interactions and the problematic concept of "cheating". The rationale for this work is well presented with a substantial introduction section and literature review. Most of the motivating examples are from plants, but the framework is applicable to any host-microbe pairing.
Thanks for your summary of our key efforts and findings, and for your assessment of our manuscript. We think you have pointed out some of our works' best strengths. Thank you.
My questions and suggestions for improvement are mostly minor.

One variant of the model investigates a scenario in which the trait is selectively neutral in the microbial partner (Fig. 2, left column). To what extent would the outcome differ if it were the host in which the trait was neutral?
We originally wondered whether to include this outcome. It is slightly different in the case of fitness feedbacks, but only because selection is more efficient in the host, so the host ends up contributing more to evolutionary change and ongoing genetic variance in the trait. As both ourselves and R2 had this specific question, and as this scenario (no direct link to host fitness), was also absent from our further simulations in the supplement, we have therefore created an additional supplementary figure that visualizes results for one example run of this scenario, combining the metrics reported in Figures 1-3 into a single figure. See new figures S2 and S3, where the figure for this scenario is S2. S3 & S2 are referenced at line 305 (end of "Simulation details", also see response to R2). S2 is also referenced at lines 339,366, & reproduced here:



In general the authors do a good job of pointing out the simplifications they made for their model and how those simplifications may affect the results (e.g., discussion of differing mutation rates, lines 226-230, generation times and dominance, line 240). One really key issue was not discussed, however: the reality that many or most host-associated microbes can survive and reproduce perfectly well without a host (e.g., free-living in soil or water). There have been some good papers recently showing that selection pressures on rhizobia differ between free-living and host-associated environments (e.g. https://doi.org/10.1111/evo.13807). Modeling the effects of this complication may be beyond the scope of this paper, however, it is an important issue that should at least be mentioned. A more general formulation of this issue is the idea of “alternative hosts” (or for the host, alternative symbionts) that will shape the evolutionary trajectory of the focal trait. In reality the fitness feedbacks between partners are probably not as symmetric as modeled here.

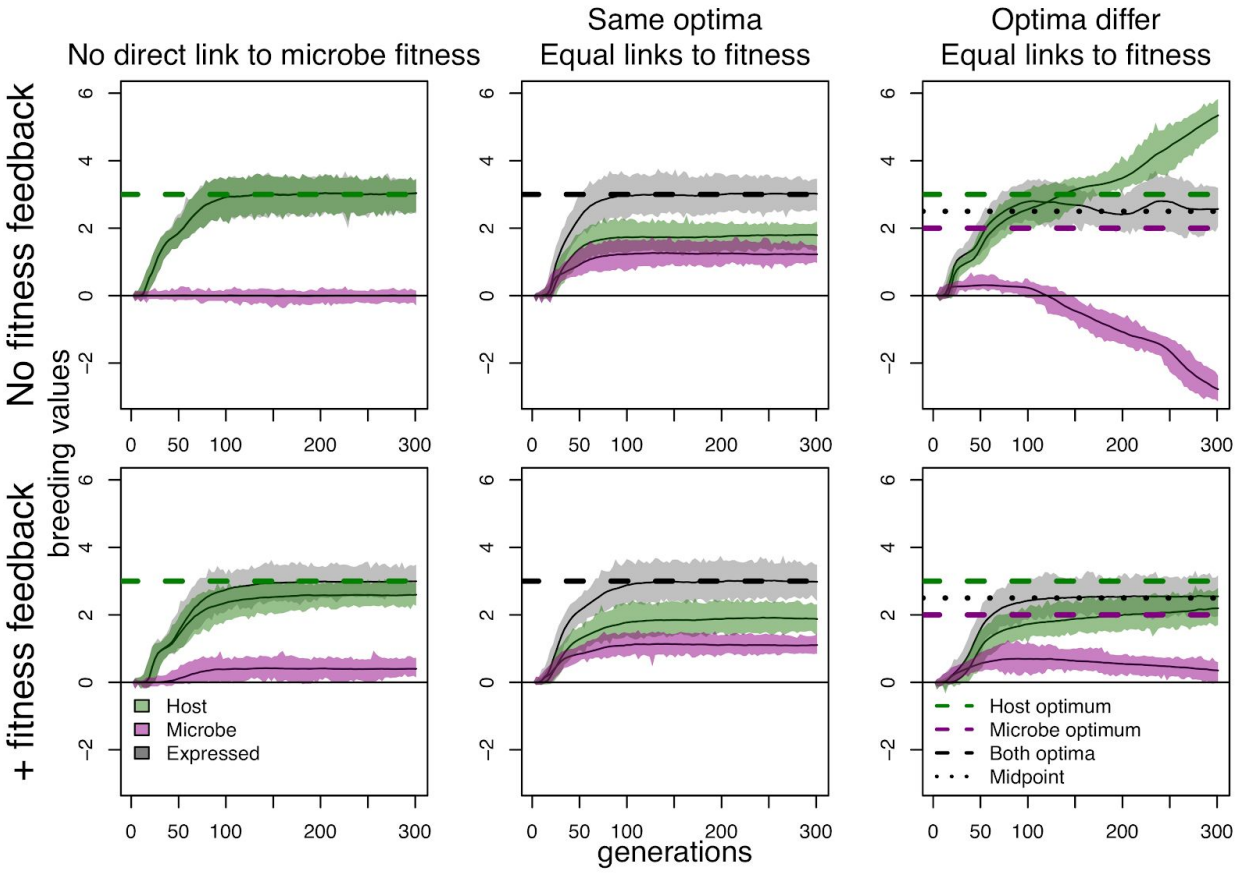
These are great questions, and indeed we had them as well. However, it is quite complex to consider phenotypic evolution in networks (e.g. Nuismer, Jordano & Bascompte 2013, cited in manuscript), or alternative selection regimes in soil. Nonetheless, our model could be viewed as implicitly including free-living or alternate host selection, if we consider the Z_{optM} and ω_M as the average lifetime fitness optima and average lifetime (direct) trait selection pressure for microbes, respectively. A treatment of either of these scenarios explicitly would require a great deal more time, effort, explanation, figures and manuscript space, and thus we think they are beyond the scope of this paper. But we agree with R2 that both would be useful, and perhaps the next steps. We have added the citation to the Burghardt et al paper to line 571 as it was a useful reference suggestion, thanks! At the same place (line 574), we now discuss temporally variable selection.

With respect to the asymmetry of feedbacks, we have already considered them, and they do indeed change the outcomes. We have now done a better job highlighting these supplemental analyses (see lines 323-327 for expanded reference to the material, Figure S8).

Line 82: I think it is premature to claim that microbes influence “most” plant functional traits. “Many” is sufficient to get the point across here. Thanks for the comment, in retrospect, we agree with R2 here -- “many” is well supported by the literature, but to claim “most” would likely require an entirely new literature review (which might not conclude that “most” plant functional traits are influenced by microbes), and is outside the scope of this manuscript. We have changed the word (line 101).

Lines 278-283: This is an excellent insight on the odd behavior often observed in eukaryotes forced to develop sans microbiota. Thank you!

Figure 2 - it is difficult to resolve the colors of the horizontal dashed lines even when zoomed in. Perhaps supplement with colored block arrows along the vertical axis to make it clearer? Upon re-inspecting this figure, we fully agree with R2 that it is difficult to see. We have increased the thickness of all lines, and changed the color of the (Midpoint) line, and used dot vs dash to distinguish from “Both optima”. We also noticed a word was missing on the top axis of this figure relative to the others (“direct”) and have corrected this (reproduced below).



Finally, we were informed that the original manuscript was a good deal too long, so we have increased the brevity throughout, and restricted the number of citations as much as possible.

These edits occur everywhere in the manuscript and are too numerous to give line numbers. In the references section of the track changes pdf, all original and added references appear, and additions/subtractions are tracked only via whether the associated number appears in the new main text. For this same reason, please also note the move of Figure 4 from the main text to the supplement (now Figure S11).

Appendix B

Dear reviewers and editors,

Thank you for carefully evaluating our manuscript. We found the new comments and suggestions useful, and have made a number of minor changes to our manuscript that we believe improve clarity, and add brief interesting discussion.

We have also cut approximately 10% of the total word count, as was requested by the editorial office to comply with page count limits.

Please see our responses in blue below, and track changes, following. Line numbers refer to the main text (not the tracked changes).

Sincerely,
The authors.

Associate Editor
Board Member
Comments to Author:
Thank you for your contribution to this special issue on Wild Quantitative Genomics. Two reviewers and I have evaluated this revised manuscript. Overall, we are positive and enthusiastic about this piece of work – we believe it to be of excellent scientific importance and interest.
Thanks for the compliments. We truly appreciate the time you've taken as editor to carefully consider our manuscript and reviewer comments, and we value your advice.

There are still some minor points to be addressed:

Reviewer 3 makes some suggestions on how the work could be better connected with terminology in multivariate quantitative genetics. This may be worth considering, or at the very least may merit some brief discussion.
We agree this point is worth considering. Please see our responses to Reviewer 3.
There are lingering issues with reproducibility. Please signpost the supplementary methods and results more clearly in the main text – point out early on in the simulated case study where the full methods can be found. In what software was the simulation implemented? Reviewer 3 also pointed out some variables that were not clearly defined.
Thanks for the catch, we have now cited R (the software for simulation) in the first sentence of the "Simulation details" section, and added a parenthetical to point out where further explanation and code may be found "further details: Supplementary Material; code: see Data Accessibility" (Lines 172-173). Please also see our responses to Reviewer 3.
I suggest revising the language in lines 98-101 – on my first read, it implied that GWAS explains a small proportion of heritable variation and that inherited microbes explains the rest (or at least a large proportion).

On re-read, we see what the editor means. We intended to convey the opposite distribution of heritable variation across sources. We have changed this to “...may help resolve the `missing heritability' paradox: since (host) loci identified via GWAS incompletely explain heritable trait variation, some causal variants may reside in genomes of inherited microbes (29; but see other likely explanations, 30)”

Reviewer(s)' Comments to Author:

Referee: 3
Comments to the Author(s).
In this paper the authors develop a novel simulation model to explore the (co)evolution of host-microbe joint-phenotypes and develop a GWAS approach to identify causal loci in multiple genomes. Overall I found the paper interesting and well written. From what I understand, the concerns of the previous reviewers have been addressed, making for a very nice paper that appears ready for publication. My only comments are on a potentially useful connection with multivariate quantitative genetics and to provide some minor suggestions for notation/terminology to help connect the model formulation with classical quantitative genetic theory.
Thanks for the compliments and suggestions, R3. We appreciate your time and effort!
Potential Connection with Multivariate Quantitative Genetics
In the formulation of their model the authors assume host-microbe pairs are drawn at random. However, it seems in general that genetic correlations between hosts and microbes across interacting pairs can form via the evolution of interaction preference or microbe inheritance. Denoting the host and microbe contributions to the joint-trait z by zH and zM respectively (so that z = zH + zM), additive genetic covariance between zH and zM can modulate the response to selection in both partners. I am therefore curious about whether multivariate quantitative genetic theory is useful for understanding the evolution of joint-phenotypes. However, this may be a contentious point since the multivariate approach might suggest that interacting partners can evolve as a single evolutionary unit. Thus, it would wonderful if the authors could briefly discuss reasons for and/or against the use of multivariate quantitative genetics to understand the evolution of joint-phenotypes.
The reviewer has both hit on an interesting point, and summarized the reasons we didn’t embark on this! For horizontally transmitted microbes, it doesn’t make sense to think of hosts and microbes as single evolutionary units (holobionts). One of the interesting things about our model is that hosts and microbes partner completely at random, so there is no opportunity for genetic correlations to arise pre-selection through partner choice, vertical transmission, or pseudo-vertical transmission (spatial structure). Even without these correlations our model generates patterns that could be measured in real data. We agree that future extensions of the model either with an explicit multivariate quantitative genetic framework and/or by introducing processes (partner choice / spatial structure) that could generate these additive genetic covariances would be very worthwhile. We now briefly expand our previous discussion of spatial structure to recommend leveraging this formulation for such cases in the future. Lines 442-447.
Suggestions for Notation/Terminology

• line 247: The variable h is not defined. I'm assuming this is the dominance coefficient, but it would be good to explicitly state this.

Thanks for catching this, we don't actually include any variation in h, so we have replaced this "h" with it's definition, "dominance coefficients". (Line 184)

• line 255: It's standard to use θ as the abiotic phenotypic optimum. Replacing zopt with θ will also reduce clutter in the subscripts later on.

We agree that this is a more aesthetically pleasing notation choice, and have substituted them. We note that there is precedent for the Zopt notation in well-cited papers, which is why we had them. For example:

Le Corre, V., & Kremer, A. (2012). The genetic differentiation at quantitative trait loci under local adaptation. *Molecular ecology*, *21*(7), 1548-1566.

• line 255: The selection parameter ω2 is often called the width of selection. However, some prefer to write A = 1/ω2 as the strength of selection since it has the intuitive property that selection becomes stronger as A becomes larger.

We agree with the reviewer that "width" and "inverse strength" of selection may be both more clear and precise than "steepness" or "inverse steepness." We have altered our language at various places in the main text and supplement, most importantly at lines 206, 260.

• eqn 2: Absolute fitness is often written as W(z) and relative fitness as w(z) = W(z)/W where W is classically absolute fitness averaged across the population. In coevolutionary models, however, W corresponds to absolute fitness averaged across trait values of potentially interacting pairs. Hence, in the context of this paper, WH corresponds to the direct absolute fitness of the host averaged across host-microbe pairs.

Equation 4 is the equation where we have a true measure of relative fitness. We therefore replaced our previous notation for relative fitness with w(z) in equation 4. Equation 2 measures a component of fitness when the corresponding (host or microbe) α is >0, we have now made this more clear. Thanks, R3 for pointing out this easy way to increase the clarity of our equation!

Also, because we rely on a simulation model with a fixed population size, sampling in accordance with the realized fitness (relative fitness, w(z), and stochastic events) of each randomly paired host and microbe is what determines the frequencies of each genotype in the subsequent generation. Therefore, we don't use or rely on host fitness averaged across possible host-microbe pairs in any equations or simulations (we don't think fitness components are the same as these), so we have not included W(z) or WH notation. We assume the reviewer included the discussion of absolute fitness and host fitness averaged across host-microbe pairs to complete their point about relative fitness. We hope we have not misunderstood the reviewer.

This comment helped us identify changes to our notation for the fitness component equations (2 and 3) that we hope clarify them. Specifically, while we vary ω and zopt (now θ) parameters in the simulations, in the context of this explanation, it seems most clear to write equations as only functions of z. We also changed Fdirect to C(z_n), which has fewer subscript letters, yet adds the indication that it is still a function of the nth expressed trait value. We hope these changes adequately address the reviewer's point. (equations in lines 191-210)

• line 256: It seems the size N corresponds to the number of host-microbe pairs. It would be good to state this explicitly especially since N is often used to denote population size.

We agree explicitly stating this could be useful. We therefore moved the note on population size to the beginning of the previous paragraph (lines 177-179), and edited it. The sentence reads “For each simulation step, we draw from the populations of hosts and microbes (both of size *N*=2,000) such that they interact at random, assuming for simplicity that each host interacts with one microbe (therefore *N* also equals the number of host-microbe pairs).”

Referee: 2
Comments to the Author(s).
The authors have done an admirable job of responding to the issues raised by both reviewers and the editor. I particularly appreciate the references added to the revised version that further clarify this paper's relationship to past work. It reinforces my opinion that this will be a very useful addition to the literature. I have no further requests for edits.
Thanks for noticing, R2 - we worked hard on this!