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Professor Inder Verma
Editor-in-Chief *Proceedings of the National Academy of Sciences of the USA*

Dear Professor Verma,

Thank you for inviting us to submit a revised version of our manuscript "Genetic specificity of a plant-insect food web: implications for linking genetic variation to network complexity" [2015-13633R] for publication in *Proceedings of the National Academy of Sciences of the USA*. We appreciate the time invested by the Editorial Board, the expert editor, and the reviewers, and have sought to incorporate their suggestions into a revised version of our manuscript. Below, we have listed the comments by the expert editor and the two reviewers in bold, followed by a detailed point-by-point response.

We hope you find the revised version to be substantially improved and suitable for publication in *Proceedings of the National Academy of Sciences of the USA*. Please let us know if you have any questions or if you need any further clarification.

Thank you for your assistance with this manuscript.

Sincerely and on behalf of my co-authors,

Matthew A. Barbour

Editor Comments:

The revision does a good job of dealing with previous comments with one very important exception. The argument of previous reviewers 1 and 3, and current reviewer 1, emphasized by me in my original instructions to them, was not taken seriously, and their response on this point is unsatisfactory. Otherwise, this paper would deserve to be published in PNAS, as it is a well-done experiment of broad significance and interest. So I will repeat:

We did take all previous comments seriously; however, we admit that we may have focused more on the comments related to the Methods instead of the comment related to the framing of the manuscript. We appreciate that the expert editor has allowed us to resubmit a revised version of the previous manuscript to address this important comment. We have done so by modifying the title as well as our wording in the Abstract, Introduction, and the rest of the manuscript so that it is now clear which results are based on simulations (details in point-by-point response below). We hope the expert editor finds that this manuscript is no longer misleading in anyway.

1. Although the evidence is empirical, the title is misleading, especially combined with the abstract, which says nothing about a simulation. I'm not great at titles, but this one must be changed. As stated, it implies that the experiment changed intraspecific genetic variation of the plant in the field, and it did not.

1. The title for the updated manuscript reads:

"Genetic specificity of a plant-insect food web: implications for linking genetic variation to network complexity"

We feel this updated title now reflects our major empirical finding (i.e. genetic specific of the plant-insect food web), but also suggests the implications of this genetic specificity based on our simulations (i.e. network complexity).

2. The first mention of a simulation is in line 462. There should be something about it in the abstract, IN ADDITION TO THE TITLE CHANGE.

2. We now mention our use of a simulation two times in the Abstract (italicized lines below):

"Theory predicts that intraspecific genetic variation can increase the complexity of an ecological network. To date though, we are lacking empirical knowledge of the extent to which genetic variation determines the assembly of ecological networks, as well as how the gain or loss of genetic variation will affect network structure. To address this knowledge gap, we used a common garden experiment to quantify the extent to which heritable trait variation in a host plant determines the assembly of its associated insect food web (network of trophic interactions). *We then used a resampling procedure to simulate the additive effects of genetic variation on overall food-web complexity.* We found that trait variation among host-plant genotypes was associated with resistance to insect herbivores, which indirectly affected interactions between

herbivores and their insect parasitoids. Direct and indirect genetic effects resulted in distinct compositions of trophic interactions associated with each host-plant genotype. *Moreover, our simulations suggest that food-web complexity would increase by 20% over the range of genetic variation in the experimental population of host plants.* Taken together, our results indicate that intraspecific genetic variation can play a key role in structuring ecological networks, which may in turn affect network persistence."

3. Throughout the manuscript, the authors should be very, very careful to show that they have only indirect evidence (though strong indirect evidence) that increased genetic variation in their system WOULD increase network complexity.

3. We have revised the manuscript very carefully and made every attempt to clarify throughout the manuscript whenever our results are based on simulations. Below, we list where we have adjusted the wording in the manuscript. Italicized portions highlight the specific adjustments we made (note that these portions are not italicized in the main text).

Lines 46-50 of the Significance section now read: Here, we *empirically* identify how genetic variation within a host plant directly and indirectly affects its associated insect food web, resulting in distinct trophic interactions occurring on each host-plant genotype. Moreover, *simulations of our empirical data suggest* that higher levels of host-plant genetic variation lead to a more complex plant-insect food web.

Lines 86-87 of the Introduction now read: "In this study, we quantify the genetic specificity of trophic interactions and *use these data to simulate the additive effects of genetic variation on food-web complexity*."

The headings within the Results and Discussion (line 166) as well as the Material and Methods (line 321) now read: "*Simulating the additive effects of* genetic variation on network complexity."

Lines 169-171 now read: "After accounting for sampling effort (dashed line, Fig. 6), *our simulations suggest* that food-web complexity *would* increase by 20% with increasing genetic variation (Fig. 6)."

Lines 193-196 now read: "It is worth noting though that our qualitative conclusion, namely that genetic variation *likely* increases food-web complexity, will still hold unless negative, non-additive effects are equal or greater in magnitude compared to the additive effect we observed."

Lines 199-200 now read: "Our results suggest that the gain or loss of genetic variation within a key species *may* fundamentally alter food-web complexity and therefore the persistence of food webs."

Lines 210-211 now read: "Indeed, our *simulations* suggest that the loss of genetic variation will result in less complex food webs."

Lines 538-540 in the legend of Fig. 6 now read: “Fig. 6. *Simulations of our empirical data indicate* that increasing willow (*Salix hookeriana*) genetic variation results in a more complex plant-insect food web due to complementarity in trophic interactions.”

Reviewer Comments:

Reviewer #1:

Suitable Quality?: No
Sufficient General Interest?: Yes
Conclusions Justified?: No
Clearly Written?: Yes
Procedures Described?: Yes

Comments:

The manuscript by Barbour et al remains a fascinating study, addressing a very important question. When I reviewed this manuscript previously, I had but one substantial complaint. Namely, the authors purported to show "empirically" that more plant genetic variation led to more complex food webs. This implies (to me, and apparently another reviewer) that the authors experimentally manipulated the combinatorics of plant genetic diversity and then empirically measured the resulting food web network structure. That would be very, very exciting. However, what the authors actually did was quite different: they measured the food webs for each of many plant genotypes, separately. They then simulated various combinations of these genotypes and calculated the expected insect food web complexity, making empirically untested assumptions about additive effects of plant genotypes. The result is a conclusion that is, strictly speaking, a theoretical prediction, albeit one generated with an
empirically parameterized model. The authors presented this work using language so ambiguous that I had to reread the paper twice to convince myself that the experiment I had thought/hoped they had done was in fact just a simulation.

4. We have taken extra care in this revised version of the manuscript to avoid ambiguous and misleading language. We hope Reviewer #1 finds that the revised manuscript is now suitable for publication.

Although both I and another reviewer raised concerns about this ambiguous use of a simulation, the authors have submitted a revision that perpetuates the same problem. Take the title:

"Intraspecific genetic variation increases network complexity: empirical evidence from a plant-insect food web"

seems to imply that they have empirical evidence (which most people would take as observational or experimental) for this effect of genetic variation, whereas in fact it is a simulation that uses empirical data as an input. This isn't to denigrate the data showing that host plant genotypes differ in their insect communities: that's a nice result that I still like. But the phrasing here focuses on the effect of genetic variance, and yet ever data point that they collected was based on single- genotypes, not on combinatorics of genotypes.

5. As discussed in response #1 to comment #1 of the editor, we have changed the title for the revised manuscript to now read:

"Genetic specificity of a plant-insect food web: implications for linking genetic variation to network complexity"

There are many other places in the text where they perpetuate this ambiguous notion of "empirical evidence" (which in their rebuttal they claim is different from experimental evidence; maybe so, but simulations are neither). For instance in the abstract:

"Moreover, we found that food-web complexity increased by 20% over the range of genetic variation in the experimental population of host plants. Taken"

6. To clarify that we did not experimentally manipulate genetic variation, we now specify that we used simulations to determine the effects of genetic variation on food-web complexity. This sentence (lines 36-38) now reads (italicized portions highlight alterations): "Moreover, *our simulations suggest* that food-web complexity *would* increase by 20% over the range of genetic variation in the experimental population of host plants."

or in the introduction:

"examine whether increasing genetic variation results in greater network complexity using a common garden experiment of a host plant"

7. To clarify that we did not experimentally manipulate genetic variation, we now specify that we used simulations to determine the effects of genetic variation on food-web complexity. This sentence (lines 87-88) now reads: "In this study, we quantify the genetic specificity of trophic interactions and *use these data to simulate the additive effects of genetic variation on food-web complexity*."

or on the second page:

"Taken together, our study seeks to test theoretical predictions for how intraspecific genetic variation influences the structure of ecological networks. "

8. To clarify for readers that we were not empirically testing any theoretical predictions, we replaced "test theoretical predictions" with "examine". This sentence now reads (lines 107-108):

"Taken together, our study seeks to *examine* how intraspecific genetic variation influences the structure of ecological networks."

I find this phrasing to be downright misleading, You have to get to line 461, for instance, before the word "simulate" shows up. The fact that the phrasing persists after another reviewer and I both voiced concerns is frustrating.

9. We apologize for not addressing the concern in our phrasing in the previous version of the manuscript. We have now adjusted our wording throughout the manuscript to avoid being misleading (please see response #6-8 to reviewer #1 as well as response #3 to point #3 of the editor).

With this in mind, I will argue strongly that the manuscript in its present form is unacceptable: it presents a bait-and-switch that implies an empirical (even experimental!) result [see the second-to-last last of the above quotes], but it delivers something entirely different. This isn't to say that the paper can't be rescued - it can. For instance in the right side of page 1, the opening sentence of this paragraph could read:

"In this study we quantify the genetic specifity of trophic interactions, and use this data to simulate the effects of genetic variation on network complexity (assuming additive effects)" This would still be interesting (though not quite as much so), but far more appropriate.

10. We hope that Reviewer #1 finds that our adjusted wording in this version of the manuscript is sufficient to rescue the paper. As suggested by Reviewer #1, we have altered the opening sentence of this paragraph so it now reads (lines 87-88): "In this study, we quantify the genetic specificity of trophic interactions and use these data to simulate the additive effects of genetic variation on food-web complexity."

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:

The authors nicely responded to all of the comments I've been in the first evaluation. Thanks for considering them satisfyingly and congratulations for a great paper!

We are happy to hear that we did a satisfactory job responding to Reviewer #2's comments.