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Dr. Inder Verma

Editor-in-Chief *Proceedings of the National Academy of Sciences*

Dear Dr. Verma,

Thank you for inviting us to submit a revised version of [2015-13633] “Intraspecific genetic variation increases network complexity: empirical evidence from a plant-insect food web” for publication in *Proceedings of the National Academy of Sciences*. We appreciate the time invested by the Editorial Board, the expert editor, and the three 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 three 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. 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's Remarks to Author:**

**This paper is promising, but I want the authors to do 2 things:**

**1. Read the reviews and then write a point-by-point response.**

**2. Revise in light of the reviews.**

**I am particularly concerned with the response to point 1 of reviewer 1, which is related to point 1 of reviewer 3. However, many of the other comments seem cogent to me and could fairly easily be accommodated in a revision.**

Suitable quality: 1,1,1

Sufficient general interest:1,1,1

Conclusions justified:0,1,0

Clearly written:1,1,0

Procedures described:0,1,1

We note that all three of the reviewers agreed that the original manuscript was both of suitable quality and sufficient general interest for publication in *Proceedings of the National Academy of Sciences*. The major issue was that 2 of 3 reviewers felt our conclusions were not justified, whereas the reviewers responses were idiosyncratic in concern to the clarity of the writing and description of the procedures. Here, we address all of the concerns raised by the reviewers, in particular, focusing on the justification of our conclusions.

**Reviewer Comments:**

**Reviewer #1:**

**Suitable Quality?: Yes**

**Sufficient General Interest?: Yes**

**Conclusions Justified?: No**

**Clearly Written?: Yes**

**Procedures Described?: No**

**Comments:**

**The notion that food web structure is altered by intraspecific trait variation has been previously proposed (in some citations in the present paper), but to my knowledge has not been clearly demonstrated. The present manuscript therefore represents a substantial advance that merits consideration for publication in PNAS. I have only a few comments after a careful reading (which is unusual, for me), highlighting that the paper is well written and fairly compelling.**

We appreciate Reviewer #1’s recognizes the merit of this manuscript, in that it represents a clear empirical example of how intraspecific trait variation influences food-web structure.

**My primary complaint is that the key result (Figure 6, showing that food web complexity increases with willow genetic diversity) is a result of in silico resampling of individual genotypes' data, rather than an empirical result in its own right. I actually wasn't entirely certain whether Figure 6 was empirical or simulated until I read the methods. Not that simulations are unacceptable: the paper is still novel in that it uses empirical data on food web structure for each of many host-plant genotypes, to draw an inference about the effects of genetic diversity. But the reader must not be allowed to confuse the two: this is NOT an experimental test of the effect of genetic variation on food web structure, just a demonstration that food web structure varies among host plant genotypes (and an extrapolation that this would lead to a food web complexity/genetic variation relationship).**

We agree with Reviewer #1 in that it is important that the reader does not confuse Figure 6 for an experimental test of the effect of genetic variation on food-web structure.

TASK: Make it clear in the beginning of this section that this is not an experimental test and that we are simply resampling individual genotypes’ data to suggest a relationship between food-web complexity and genetic variation.

**Of course, the simulated results are a nice use of the empirical data, but they must not be confused with empirical reality; there may be non-additive effects of host plants arising from interactions between species supported by (or inhibited by) particular host plants. Host plant genetic variation can cause dilution effects, or allee effects, or subsidies that sustain a given insect species on all host genotypes that would otherwise be sustainable only on certain host genotypes (e.g., source-sink dynamics). I could go on listing hypothetical ways in which host plant genotypes may not have additive effects on the food web. The essential point is that the authors have made assumptions when generating the simulated food webs, to test the effect of genetic diversity on food web complexity. Those assumptions are not spelled out clearly enough for me to evaluate, nor are they likely to be rock-solid. So I, for one, will view Figure 6 as both the most interesting and least convincing**

**result in the paper.**

We recognize that non-additive effects of host-plants on food-web structure may arise in a variety of ways. Indeed, we did mention in our original submission that “QUOTE”. Nevertheless, we agree with the reviewer that the assumptions of this procedure were not presented clearly enough in the original manuscript, although we did mention that this procedure could only predict the additive effects of genetic variation on food-web structure.

We would like to address Reviewer #1’s comment about these assumptions not likely to be “rock-solid”, in that we hope that this interesting relationship is still convincing.

TASK: Identify primary assumptions of this procedure (i.e. additivity of interactions). Review prior literature on biodiversity-ecosystem function relationships to address ensure that I’m accurate in the assumptions of this model.

TASK: Review papers of genetic diversity relationships with arthropod response variables (examine recent meta-analysis in Journal of Ecology), to see document how frequently responses were additive or non-additive, and if non-additive, the direction of the non-additivity (e.g. negative or positive effects on arthropod richness). This will provide an assessment of whether this analysis is likely an underestimate (hoping for this) or an overestimate of the effects of genetic variation on food-web structure.

**A second, relatively minor, concern is the statement that the authors have identified "the traits mediating the genetic specificity" (line 259). Rather, they have found correlations between a few traits and the host plant genotypes' ability to support particular trophic interactions. There is only correlative support (hence the phrase 'associated with changes in the abundance of galling insects" (line 263)), and so I found the phrase "determine the traits mediating..." to be a bit too strong and implies false certainty about cause and effect, without suitable experimental or genetic manipulations of traits to rule out correlated traits arising from pleiotropic effects or other forms of co-inheritance.**

Again, Reviewer #1 brings up an important point. On the one hand, there is considerable support from the plant-herbivore literature that plant traits are important determinants of herbivore phenotypes, and abundances (and therefore community composition). Indeed, despite our exhaustive trait measurements (40 traits reduced to 12 uncorrelated axes of variation ), there may be other important traits that we did not measure, such as phenology, that could account for these relationships. Indeed, we point this out in a previous manuscript that examined how genetic variation influences herbivore community composition via phenotypic trait variation (Barbour et al. 2015, Functional Ecology). Accordingly, we have modified our strong inferences to imply more correlative support and as interesting hypotheses worth testing.

TASK: calculate maximum correlation among 12 axes of predictor variables.

TASK: tone down language such as “determine” and “mediating” and “identifying traits”, to “associations” and “correlations”, etc.

References:

Barbour et al. 2015, Functional Ecology reference

**Following up on the last point above (co-inheritance), because the present study is just sampling wild willow genotypes from a natural population, nothing is (it seems) known about genetic relatedness among the genotypic accessions studied here. To what extent are these genotypes really independent? Or, are some pairs of genotypes really clonal variants, or siblings, or in other ways more closely related? This is a nit-picky point that should not be used to reject the paper. But, the authors should remind the readers that relatedness among genotypes is not known (unless I am wrong on that point, in which case relatedness should probably be a covariate in the analysis, typically in the form of a known error matrix in a generalized least squares model). This relatedness can, if substantial, undercut the independence of sampled genotypes and introduce potential spurious confidence in correlations between genotype' traits (including their species interactions).**

Reviewer #1 is correct that the relatedness among genotypes is not known, and we hope that this point is not used to reject the paper. The matrix of microsattelite markers was published in the supplementary material of Barbour et al. (2015, Functional Ecology), but since the genotyping was only based on 2 markers, we felt uncomfortable in trying to infer the relatedness of these genotypes. Indeed, one of the genotypes was removed completely from all further analyses associated with this project because it was identified as being a redundant.

In terms of the independence of these genotypes, another way to examine this is by examining the phenotypic trait variation among genotypes in multivariate trait space.

TASK: RDA ordination and visualization of genotypes in multivariate trait space. Base this off the samples used for this paper.

**Figure 3C - the y axis is scaled to be identical to Figure 3A and 3B, with the result that all the interesting variation is invisible, if any. I suggest rescaling the axis so that the variation in the plotted data is visible. The authors could just state clearly that y axes are not identical, to avoid readers getting confused. Same goes for Figure 4B and 4C. Furthermore, much of the vertical variation in this figure (and Figure 4) is taken up by a few outlier points. Using a log scale on the y axis can help the readers focus on the variation in means, rather than the location of outliers.**

TASK: create these visualizations as suggested by the reviewer, then decide which ones to include in the paper.

**Line 530-533 states that the dashed line in Figure 6 is the 'expectation for sampling effects alone', in relation to "the average complexity of food web in polycultures'. However, this is misleading. The dashed line is the maximum individual-genotype food web complexity, which is GREATER than the expectation for the average (for N genotypes in combination) of the sampling effect alone, because for a sample of N genotypes one may or may not sample that most-complex single genotype. The authors could use a sampling procedure to generate a more sophisticated null expectation for sampling effects that better accounts for (1) variance in genotype-specific food web complexity, and (2) sampling of those genotypes. This null expectation for the mean would tend to lie below that dashed line.**

Reviewer #1 brings up a good point in that our expectation for sampling effects alone was a conservative estimate.

TASK: review how biodiversity-ecosystem function literature to identify a more appropriate null expectation for sampling-effects.

**Reviewer #2:**

**Suitable Quality?: Yes**

**Sufficient General Interest?: Yes**

**Conclusions Justified?: Yes**

**Clearly Written?: Yes**

**Procedures Described?: Yes**

**Comments:**

**This manuscript reports the investigation of the effect of genetic variation of the plant Salix kookeriana on the composition and abundance of associated insect galls and their parasitoids. The study is based on an impressive common garden experiment of 26 different willow genotypes, each replicated 25 times. The experiment and the analysis are well-done and convincing, and the manuscript is well presented. I really enjoyed performing this evaluation.**

We appreciate Dr. Gravel’s (Reviewer #2) recognition of the quality of this manuscript.

**I found two results that are particularly interesting:**

**The effect of host genetic identity propagates up the food chain and indirectly affect higher trophic levels. There is already evidence in the literature there is strong genetic variability among plants in their resistance to herbivory, and some have also looked at the composition of associated herbivores. But this study is the first, to my knowledge, to study the impact of the host identity on the enemies of the associated herbivores. The experimental design is quite clever and allows this unique kind of analysis.**

Again, we agree with Dr. Gravel in that this is at least the first study to examine the impact of host-genotype identity on a species-interaction network of its associated community.

**The authors not only document the difference of the insect community found on the leaves, they also investigate the traits driving these interactions. This analysis provides a better understanding of the mechanisms driving the variability among hosts. It will also make basis for predicting the action of natural selection on both the host and the herbivores.**

Again, we appreciate that Dr. Gravel recognizes the importance of including the detailed analysis of plant traits, as we believe this is the underlying mechanism determining the plant-herbivore interactions, and that this would enable us, at least on the first hand, to predict how natural selection may influence the structure of this interaction network.

**I only have a few comments, which should be viewed mostly as constructive suggestions:**

**- We have to believe the authors that there is significant genetic variation of traits among the host genotypes until we get to the methods. I got quickly convinced of it reading the second paragraph of the methods. It would perhaps make the story more convincing if early in the results the authors could mention the amount of trait variation there is among the genotypes, and which traits do vary the most.**

TASK: Consider putting some of the trait variation earlier on. This is difficult, because this would just be reviewing old results, but perhaps it would make things more clear from the beginning.

**- The ordination of the insect community on the different hosts is only provided in the last figure (6), while the associated statistical test is presented in the first paragraph of the results. I would bring this figure up front in the ms.**

TASK: Technically, the associated statistical test is presented later on, as this is an ordination of entire food web, plant-herbivore and herbivore-parasitoid interactions associated with each genotype. Since this is what Figure 6 is based on, we feel that it is best to keep this ordination embedded within Fig. 6.

**- I understood from the methods that each point in the Fig. 6 is the predicted LD from the multivariate GLM. My feeling is that doing it this way, the figure underestimate the amount of variability since it is the result of fitted models and not the original data. Instead, I would sample the data directly for S genotypes (the sensitivity to the number of replicates has to be evaluated) and compute LD from the original data instead of the fitted models.**

TASK: Determine an appropriate resampling procedure that is in line with Dominique’s comments.

**- Same figure: in addition to the number of genotypes, I would plot the LD as a function of functional diversity. According to the interpretation, the relationship should saturate much slower.**

We are not entirely clear what the Dr. Gravel means here. Perhaps we could investigate plotting this relationship with the functional trait diversity of the genotypes.

TASK: look into methods for quantifying functional trait diversity. May have to do this for the traits that were important as well as the ones with all of the traits included.

**- At my second time going into the manuscript, I got stuck on the first sentence of the intro: while we do understand the effect of the network complexity at the community level on the dynamics of ecological networks, there is a big gap in the theory to address the impact of complexity within a population. We could not simply translate theory conducted at the community level to make prediction at the population level. Genotypes are not equivalents of species when looking at the dynamics since the entire population contributes to reproduction. We currently have no theoretical understanding of what are the impacts of genetic diversity on network dynamics. This manuscript therefore opens a new research agenda, not only for further empirical investigations, but also for theory. This gap of knowledge should be highlighted somewhere in the conclusion.**

We appreciate Dr. Gravel’s recognition that this manuscript opens a new research agenda for both empiricists and theoreticians.

TASK: Highlight this gap of knowledge somewhere in the conclusion.

**I signed my evaluation**

**Dominique Gravel**

**Reviewer #3:**

**Suitable Quality?: Yes**

**Sufficient General Interest?: Yes**

**Conclusions Justified?: No**

**Clearly Written?: No**

**Procedures Described?: Yes**

**Comments:**

**This paper is very interesting in that it seeks to establish a genetic basis to the interaction network of a small community of interacting species occupying 3 trophic levels (1 willow, 4 galling insects, and 6 species of parasitoids). Using a common garden with 26 different willow genotypes, they show that different genotypes of the willow support different abundances of the gall makers, which in turn are differentially parasitized. Largely though the combined effects of differential willow resistance, which affects the abundances of the gall makers and gall thickness, which affects the oviposition success of the parasitoids, they find that different plant genotypes support different interaction networks among the 11 interacting species that are plant genotype specific. Conceptually, this is important because the genetic basis of such networks has important ecological, evolutionary, and conservation implications. The genetic basis of network structure is important for**

**understanding the interface between ecological and evolutionary dynamics in real ecosystems. The study is also novel in that it integrates genetics, trophic interactions, network analyses and community ecology using a common garden experiment. These are all important accomplishments; this is a great system, working at the interface of ecology and evolution in a real community.**

**The manuscript could be significantly improved by addressing the following points:**

**1. The title: "empirical evidence" that genetic variation increases network complexity is misleading. While I am comfortable with the finding that different genotypes support different networks of interactions, I am not convinced that the simulation using data from willows randomly planted in a common garden provides critical empirical evidence that network complexity increases with genetic diversity. In previous studies by Crutsinger et al. 2006, experimental plots were created that differed in genetic diversity, which experimentally showed that increasing genetic diversity in the plants increased arthropod diversity. Other studies have also experimentally demonstrated this relationship. To say that increased genetic diversity results in greater network complexity, it is essential that an "empirical" test perform a similar field experiment and a simulation does not meet this requirement, especially if it is published in such a high profile journal. The simulation is consistent with the hypothesis, but it is at best a weak empirical finding that several other studies have also predicted.**

We agree with Reviewer #3 in that the simulation using data from our willow common garden does not provide ‘critical’ empirical evidence that network complexity increases with genetic diversity. Indeed, one would need an experiment design, such as described in Crutsinger et al. 2006, that manipulates genetic diversity and examine the corresponding relationship with food-web complexity.

**2. Lines 60-62 - The authors claim that previous studies have not quantified how genetic variation affects the composition of pairwise interactions that determine network structure is an oversimplification. E.g., Mooney et al. (2011) has shown that the genetics based interactions of aphids and ants affect an associated community (Arthropod-Plant Interactions 5:1-7). See also Moreira & Mooney (2013. Biology Letters 9:20130133 and other studies from the same group. Another relevant study by Lamit et al. (2015. J. of Ecology 103: 840-850) showed the paired networks among 7 different communities from lichens to arthropods that varied as a function of plant genotype.**

TASK: refine Lines 60-62 to not be such an oversimplification. This may be as simple as just describing the community level effects of trophic interactions.

**3. Lines 64-66 - The authors claim that others have examined simple tri-trophic interactions, but again, the jump is incremental with 4 herbivores and 6 parasitoids. These willows support many more species from different trophic levels including mammals, birds, fungi and other arthropods that are not included in the present study. It is important to be more realistic in such claims and tone it down, as the examined community is still a relatively simple one.**

TASK: consider toning down the claim, or at least highlighting that this is still a relatively small community.

TASK: punch back on the “incremental” jump. It was a considerable task to quantify these trophic interactions for a relatively close module of a network. Indeed, this may be the most appropriate scale to look at if we want to examine quantitative variation in food webs on realistic temporal and spatial scales.

**4. It seems that these first 3 points make unnecessary claims that detract from the real accomplishment that different genotypes support different interaction networks, which represents the real accomplishment.**

**5. Lines 396-398 - Where does the potential of gall-parasitoid interactions come from? The number of parasitoids is 6 and the number of galls is 4, so the number of potential interactions would seem to be much greater than 12?**

TASK: look at where Reviewer #3 got this number of 12. Did I say that somewhere. May need to discuss that we are assuming that the trophic interactions present were constrained by the phylogenetic or interspecific trait differences, which is not the focus of this study. Perhaps I could look at rarefaction curves for the species richness of each gall species’ community.

**6. Line 463 - Most studies in such high profile journals would have more than a single season of data. How repeatable might this be a 2nd year of studies and would the networks shift? The major implications of these findings are probably only applicable if they remain relatively consistent.**

Actually, we disagree with Reviewer #3 in that the major implications of these findings are probably only applicable if they remain relatively consistent. Host-plant genetic variation may create a template of variability, which has often been shown to be an important stabilizing factor). So even if the genotype relationships varied between years, if the amount of variability was maintained, then we would predict that we would see the same relationship. Indeed, there may be important GxE interactions between years (address Peter Price’s work), but this doesn’t nullify the conclusions of this work. For example, in harsh environments, perhaps genotypic variation doesn’t matter at all, that still doesn’t nullify the expectation that genetic diversity would lead to increased food-web complexity. This would only be an issue if genetic diversity reduced food-web complexity.

If they were to vary, this would indeed make it more difficult to predict eco-evolutionary dynamics.

TASK: Examine correlations in gall abundances among years. Look at correlations in parasitoid community composition among the 2 years of data for which I have for 10 of the genotypes. Look at consistency in the composition of the parasitoid community on Iteomyia over the last 3 years.

**7. Lines 530-537 - Seems that a major conclusion based on a simulation does not set a very high bar as increasing numbers of studies are based on actual experiments in which genetic diversity is manipulated in blocks within a common garden to address such questions: E.g., Bangert et al. (2013. Restoration Ecology, 21:447-456).**

TASK: after addressing similar comment from Reviewer #1, see if this address this comment.

**8. Lines 588-590 - To say that these traits determine resistance is implying causality that only further experiments can actually confirm, such as the silencing of genes associated with these traits. More accurately, they are correlated or associated. Need to build a much stronger case that these traits are as important as you suggest.**

TASK: after addressing similar comment from Reviewer #1, briefly recount how we addressed this.

**9. The occurrence and abundance of the galling insects is to a large extent determining the frequency of interactions between galls and parasitoids. This raises the question: to what degree is the network complexity driven by the abundance of galls? The authors do test for the effect of genotypic variation on gall-parasitoid interactions as well as trophic interactions in the plant-insect food web (i.e., tri-trophic interactions); however, this test doesn't separate out the effect of gall abundances on parasitoid interaction frequency. This would be important for readers to elucidate whether or not network complexity is primarily arising from variation in gall abundances. A structural model approach (i.e., path analysis or structural equation model) would allow for the separation of direct and indirect effects of genotype on total abundance of galls and frequency of gall-parasitoid interactions and the weighted linkage density metric. In short, network complexity is not decoupled from gall abundance. This is an important feature that would add clarity to this study.**

Note that we did do this to an extent by examining variation in the probability of gall-parasitoid interactions (i.e. per-capita interaction strength) with the binomial GLM models. These suggested that while gall abundance likely explains the response for the least abundant gall species, they likely don’t explain the interactions for the most abundant.

TASK: figure out how to also track gall abundance and frequency of gall-parasitoid interactions for the simulation analysis.

TASK: Conduct path analysis that removes the effect of gall abundance. Genotype diversity -> Gall abundance -> weighted linkage density. Do I include frequency of gall-parasitoid interactions? Do I conduct this just for the averages of the 26 genotypes, or for each replicate of the simulation?