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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.

We are encouraged to hear that the expert editor feels that the paper is promising and that all three of the reviewers agreed that the original manuscript was both of suitable quality and of sufficient general interest for publication in *Proceedings of the National Academy of Sciences*. It seems that the major issue was that 2 of 3 reviewers felt our conclusions were not justified, whereas the reviewer's responses were idiosyncratic in concern to the clarity of the writing and description of the procedures. Below, we give a point-by-point response to each of the reviewer's comments. We feel that the manuscript has been much improved by incorporating the their comments.

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.

1. We appreciate Reviewer #1's recognition that this manuscript 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).

2. We agree with Reviewer #1 that it is crucial that the reader does not confuse Figure 6 for an experimental test of the effect of genetic variation on food-web structure. We make this point clear in the revised the text of the *Results and Discussion* that precedes this result.

Lines 167-169 now read: "**Intraspecific genetic variation increases network complexity.** To examine this, we used our empirical data to simulate how the complexity of the plant-insect food web would change across different levels of willow genetic variation (*Materials and Methods*)."

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.

3. We appreciate that Reviewer #1 recognizes that the simulation was a nice use of the empirical data. We also agree that the assumptions underlying our computer simulation were not presented clearly enough in the original manuscript. We have revised our simulation based on comments from Reviewer #2 (see point #13) and we have clarified these assumptions in the revised manuscript. In short, since our simulation is resampling our empirical data, we are only estimating the additive effects of genetic variation on food-web complexity. Below, we outline where in the manuscript we have made this more clear.

Lines 348-350 of the *Materials and Methods* now read: "This resampling procedure is analogous to methods used in experimental studies (e.g. 27, 28) to estimate the expected additive effects of genetic variation on arthropod diversity."

Lines 187-198 of the *Results and Discussion* now read: "An important limitation of our simulation and experimental design is that we were unable to estimate the extent to which food-web complexity is influenced by non-additive effects of genetic variation. Non-additive effects may arise in a variety of ways (e.g. competition and facilitation, associational resistance/susceptibility, source-sink dynamics), and prior work has shown that host-plant

genetic variation can have positive (25), neutral (28), or negative (39) non-additive effects on the diversity of upper trophic levels. Future experiments are needed that explicitly manipulate levels of genetic variation and test for the presence and magnitude of non-additive effects on food-web structure. It is worth noting though that our qualitative conclusion, namely that genetic variation 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."

In addition, an assumption we had implicitly made in the previous manuscript was that monocultures and polycultures had similar sampling effort. However, this is not true, as sampling effort (i.e. number of individual plants) necessarily increases with increasing genetic variation. This is an inherent constraint of our empirical data. For example, if we had measures of trophic interactions on 25 replicate plants of each willow genotype, we could directly compare monocultures of 25 plants with 25-genotype polycultures of 25 plants (i.e. 1-plant replicate of each genotype). Below, we outline how we address this issue. In short, we believe the method we describe below actually gives an overestimate of food-web complexity in monocultures. Therefore, the response in food-web complexity we now report (20% increase) is likely a conservative estimate of the additive effects of genetic variation.

Lines 170-171 of the *Results and Discussion* now read: "After accounting for sampling effort (dashed line, Fig. 6), we found that food-web complexity increased by 20% with increasing genetic variation (Fig. 6)."

Lines 352-367 of the *Materials and Methods* now read: "As is though, our resampling procedure is unable to control for the inherent increase in sampling effort with increasing genetic variation (e.g. N = 4 plants for monocultures, N = 100 plants for polycultures of 25 genotypes). Not accounting for sampling effort will give us an overestimate of the effect of genetic variation on food-web complexity. To account for this bias, we used our resampling procedure to generate 1,000 estimates of average complexity for monocultures based on progressively higher levels of sampling effort (1 – 4 plants). We then used an asymptotic model (42) to predict the average complexity of food webs in 100 plant monocultures (details in supplementary information). While more sophisticated and accurate models have been developed to extrapolate species richness (reviewed in 43), nothing has been developed for extrapolating food-web complexity. In the supplementary information, we demonstrate that our asymptotic model likely overestimates the average complexity of monocultures by about 5%. However, using this extrapolation as a baseline will still give us a more accurate (although now conservative) estimate of the additive effects of genetic variation on food-web complexity."

Lines 57-97 of the supplementary information now read: "**Asymptotic model -** For our asymptotic model, we used a scaled and shifted Michaelis-Menten function (4) of the form, $LD_{q,N_m} = \frac{a(N_m-1)}{(b+(N_m-1)} + \overline{LD_{q,1}}$,
where N_m is the number of plants in monoculture, $LD_{q,Nm}$ is the complexity at N_m, a and b are phenomenological parameters that scale $LD_{q,Nm}$ and N_m, respectively, and $\overline{LD_{q,1}}$ is a constant parameter, representing the average complexity for 1-plant monocultures. Adding the constant, $\overline{LD_{q,1}}$, and subtracting the constant, 1, shift the function so that when $N_m = 1$, $LD_{q,N_m} = \overline{LD_{q,1}}$.

We used non-linear least squares to estimate parameters *a* and *b*. Our asymptotic model appeared to provide a good fit to the data (R^2 = 0.88, $LD_{q,N_m} = \frac{0.62(N_m-1)}{(3.62+(N_m-1)} + 1.25$) and predicted a value of 1.84 for the complexity of 100 plant monocultures ($LD_{q,100}$ = 1.84).

To examine whether this asymptotic model was appropriate for our data, we applied it to the results of our primary simulation (data presented in Fig. 6 of main text). Specifically, we replaced N_m with N_G, the number of genotypes sampled, and $\overline{LD_{q,1}}$ is the average complexity for genotype monocultures, and re-estimated the scaling parameters *a* and *b*. We found that this model provided an excellent fit to our data (R^2 = 0.96, $LD_{q,N_G} = \frac{0.76(N_G-1)}{(2.23+(N_G-1)} + 1.52$). Indeed, the predicted complexity of 25-genotype polycultures was 2.209, which only deviated less than a tenth of 1% from the average calculated from our resampling procedure ($LD_{q,25}$ = 2.208). We also tried fitting non-asymptotic models (5) to our data; however, we found that both a log-linear (R^2 = 0.89, $LD_{q,N_G} = 0.19 * \log(N_G) + 1.65$) = and log-log ($R^2$ = 0.87, $\log(LD_{q,N_G}) = 0.10 * \log(N_G) + 0.50$) model had relatively low R^2, highly biased residuals, and overestimated food-web complexity by 2 and 3%, respectively, compared to the asymptotic model (predicted $LD_{q,25}$: log-linear = 2.26; log-log = 2.28).

While the above analysis suggests that our asymptotic model provides a good fit to our data, it does not give much insight into how accurate the model's predictions will be when we extrapolate beyond the original data. One way we can examine this is by refitting our model with smaller fractions of our data and seeing how accurately it extrapolates to predict the complexity of 25-genotype polycultures. When we did this, we found that the model began to increasingly overestimate food-web complexity. For example, with the first 40% of the data (i.e. 1 to 10 genotypes), the model overestimated food-web complexity by less than 1%; however, with the first 12% of the data (e.g. 1 to 3 genotypes), the model overestimated food-web complexity by about 3%. While these predictions are still quite accurate, our asymptotic model for monocultures is extrapolating based on 4% of the potential data (4 of 100 plants). Therefore, it seems reasonable to suggest that the predicted complexity of 100-plant monocultures may be overestimated by ~5%. So while this asymptotic model likely gives a more accurate baseline for estimating the additive effects of complementarity, it also suggests that the reported effect of 20% is a slightly conservative estimate."

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.

4. Reviewer #1 is correct and we have now toned down our strong inferences to imply just correlative support.

Lines 124-131 of the *Results and Discussion* now read: “Importantly though, our extensive screening of willow phenotypes (*Materials and Methods*) enabled us to identify traits that may be mediating the genetic specificity of trophic interactions with galling insects. In particular, we found that leaf C:N, certain leaf secondary metabolites (flavanones/flavanonols PC1), and plant size were associated with changes in the abundance of galling insects (multivariate GLM, $\chi^2_{3,104} = 28.44$, $P = 0.004$; Table S2), whereas leaf gall diameter was associated with variation in a different suite of leaf secondary metabolites (salicylates/tannins PC1 and flavones/flavonols PC1)(weighted linear model, $F_{2,59} = 8.27$, $P < 0.001$; Table S2).”

In addition, we toned down our strong inferences in Line 239-241 and Lines 546-547.

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).

5. Reviewer #1 is correct that the relatedness among genotypes is not known. As Reviewer #1 points out, closely related genotypes may have similar phenotypes, which would introduce spurious confidence in associations between willow traits and the abundances and sizes of galls. We address this comment in the revised manuscript by: (1) notifying the reader that the relatedness among genotypes is unknown; and (2) quantifying the functional diversity of the 26 willow genotypes in multivariate trait space.

Lines 229-232 of the *Materials and Methods* now read: “While relatedness among these genotypes is unknown, their multivariate phenotypes are quite distinct from each other (details in supplementary information), suggesting we can treat them as independent from one another.”

Lines 24-31 of the supplementary material now read: “**Relatedness and functional-trait diversity of willow genotypes –** The matrix of microsatellite markers for the 26 willow genotypes used in this study was published in Table S1 of (1); however, since the willow genotyping was only based on 2 markers, they were unable to infer the relatedness among genotypes. If certain genotypes are more closely related to each other, and consequently have very similar phenotypes, this could introduce spurious confidence in our associations between willow traits and gall abundances/phenotypes. We can examine this phenotypic similarity by measuring the functional evenness and divergence of the 26 willow genotypes in multivariate trait space (2). To do this, we calculated the average trait value for each of the 40 traits we measured for each willow genotype. We then calculated functional evenness and functional

divergence using the '*FD*' package in R. For both indices, values close to zero correspond to functional redundancy, while values close to one indicate functional distinctiveness. We found that functional evenness and divergence were equal to 0.94 and 0.87, respectively, suggesting that the multivariate phenotypes of each genotypes are quite distinct from each other. Therefore, we argue that not knowing the relatedness among the 26 genotypes probably introduces little bias in our trait associations with the abundances and sizes of galls."

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.

6. Our original intent with Figures 3A-C and 4A-C was to preserve the scale because we wanted to make it easier for the reader to clearly see which species were the dominant ones in the community, while still preserving the scale of the raw data. We agree with the reviewer that allowing the y-axes to scale differently can still illustrate that certain species and interactions are more important (via looking at the maximum value on the y-axis) as well as enabling the reader to more clearly see the variability among genotypes. Therefore, we have revised Figure 3A-C and 4A-C in the manuscript so they are each scaled differently.

We did not, however, decide to log-transform the data (or in this case log(x+1) to account for zero values). We decided against because we feel it is easier to interpret the data on the original scale, rather than log(x+1).

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.

7. In retrospect, it has become clear that our simulation is only estimating sampling effects (i.e. additive effects) of genetic variation on food-web complexity. This is because food-web complexity is a composite variable and will only increase with genetic variation if genotypes host complementary trophic interactions (i.e. addition of unique interactions and/or increasing evenness of interactions). Therefore, our baseline of comparison is simply the average complexity of monoculture food webs. Indeed, as we mentioned in point #3, our simulation is directly analogous to methods used in experimental studies to estimate the contribution of

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Comment [1]: Return to this

additive effects to arthropod diversity. However, to clarify the processes by which genetic variation increases food-web complexity, we have now implemented a structural equation model (details in point #25).

Other methods have been developed for Biodiversity-Ecosystem Function experiments, but these are only applicable in an experimental setting on univariate ecosystem responses (e.g. plant productivity: Loreau and Hector 2001, Nature; Fox 2005, *Ecol. Lett.*; Fox 2006, *Ecology*),

making interactions more even has two sources of additive effects (unlike plant productivity). Specifically, with increasing genetic variation there will be an ", This is because food-web complexity, unlike plant productivity, is a composite variable, and as a consequence, will increase with the addition of complementary trophic interactions. Specifically, in our simulation, increasing genetic variation will only increase food-web complexity via an increased probability of sampling genotypes with complementary sets of trophic interactions.

our simulation is only able to estimate the can only measure the additive effects of genetic variation, the revised manuscript, we have modified our approach to estimating Reviewer #1 is We have revised our simulation methods at the recommendation of Reviewer #2 (see point #_ for details). Now, our simulation is similar to the ones that have been used in experimental studies to determine the expected contribution of additive effects of genetic variation on insect diversity (Crutsinger et al. 2006, *Science* ; Johnson et al. 2006, *Ecol. Lett.* ; Crawford and Rudgers 2010, ___). Additive effects of genetic variation on composite indices (e.g. diversity, food-web complexity) may be due to: (1) increased probability of sampling genotypes with more complex food webs; and/or (2) increased probability of sampling genotypes with distinct food webs (i.e. complementary). To our knowledge though, no methods have been developed to tease apart the contribution of these two additive effects and it is not immediately clear to us how we would do this in our study. In the revised manuscript, we have removed the dashed line from Figure 6 and use our ordination of trophic interactions (inset of Figure 6) to show that the positive relationship between genetic variation and food-web complexity is due in part to genotypes hosting complementary trophic interactions. Lines _-_ now read,

“”

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.

8. 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.

9. Again, we agree with Dr. Gravel in that, to our knowledge, this is the first study to examine the direct and indirect effects of host-plant genotype on a species-interaction network.

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.

10. We appreciate that Dr. Gravel recognizes the importance of including the detailed analysis of plant and gall traits to better understand the mechanisms mediating these trophic interactions. While both Reviewer #1 and #3 point out that we can only infer correlative support for these mechanisms, we feel that analyses such as ours are an important first step toward predicting how natural selection may influence the structure of interaction networks.

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.

11. Dr. Gravel brings up a good point. We have now made it clear in the introduction that there is substantial heritable phenotypic variation in this system that is associated with resistance to galling insects. Lines 89-93 now read:

"First, we have demonstrated in previous work that *S. hookeriana* (hereafter, willow) displays heritable variation in traits associated with leaf quality (36 traits, mean $H^2 = 0.72$) and plant architecture (4 traits, mean $H^2 = 0.27$), some of which are also associated with resistance to its community of galling herbivores (19)."

- 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.

12. Technically, the associated statistical test is presented later on (Figure 6's legend), as this is an ordination of the entire food web, plant-gall and gall-parasitoid interactions, associated with each willow genotype. Since this ordination is crucial for our interpretation that complementarity in trophic interactions among genotypes contributes to the positive relationship between genetic variation and food-web complexity, we feel that it is best to keep this ordination embedded within Figure 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.

13. Dr. Gravel is correct that our prior method for the simulation underestimates the amount of variability in LD (food-web complexity) at different levels of genetic variation. As suggested, we revised our simulation methods to directly sample from the original data. We also replicated this simulation 40 times to estimate how sensitive our simulation was to different subsamples of our data. Below, we outline the updated methods.

Lines 329-350 in the *Materials and Methods* now reads: "To examine whether genetic variation increases food-web complexity, we designed a resampling procedure to estimate the complexity of the plant-insect food web at different levels of genetic variation (range = 1 to 25 genotype polycultures) from our empirical data. We omitted 1 of the 26 genotypes from this analysis (Genotype U) because we never found any galls on the branches we sampled. Our resampling procedure consisted of the following two steps. (1) Generate quantitative matrices: In order to ensure willow genotypes had equal sampling effort, we randomly sampled 4 individual willows of each genotype (without replacement) and their corresponding trophic interactions (willow-gall and gall-parasitoid). Next, we calculated the total abundance of each trophic interaction associated with each genotype, resulting in a quantitative matrix of 25 genotypes (rows) and 16 unique trophic interactions (columns). (2) Sampling genetic variation: with this matrix, we randomly sampled 1 to 25 genotypes (without replacement), 1000 times each, and calculated the total abundance of each trophic interaction associated with each level of genetic variation. We removed redundant combinations of genotypes that were generated by our random sampling. We then calculated food-web complexity (described at end of this section) for each sample, and then calculated the average complexity for each level of genetic variation. Finally, we repeated this

sampling procedure on 40 different matrices to quantify the variability in our estimates of average food-web complexity."

- 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.

14. While we agree this would be very interesting to do, we feel that perhaps adding in 'functional diversity' would be distracting as this is not a concept that we have adequately developed throughout the paper.

- 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.

15. We appreciate Dr. Gravel's recognition that this manuscript opens a new research agenda for both empiricists and theoreticians. We now highlight this point in our conclusion.

Lines 216-218 now read: "At this point though, we are currently lacking a theoretical and empirical understanding of how genetic variation scales up to affect the dynamics of food webs."

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.

16. We appreciate Reviewer #3's recognition of the quality and interdisciplinary nature of this manuscript.

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.

17. 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 experimental design, such as described in Crutsinger et al. (2006, *Science* 313:966-968), that manipulates genetic diversity and quantifies the corresponding response in food-web complexity. This is actually the reason why we used 'empirical' instead of 'experimental' for the title of the original manuscript, since our simulation of the relationship between genetic variation and food-web complexity was based off empirical data. Still, it is not our intent to be misleading in anyway; therefore, we have removed 'empirical' from our title so it now reads:

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

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.

18. We have deleted this claim from the revised manuscript. Note that we address this comment as well as the other 'unnecessary claims' more fully in point #20.

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.

19. We have deleted this claim from the revised manuscript. In addition, we make it clear to the general reader that our food web represents a distinct compartment of the larger food web associated with willows. Lines 93-99 now read:

"Second, the unique biology of galling insects makes them ideal for building quantitative food webs. In particular, galls provide a refuge for larva from attack by most generalist predators (20); therefore, galls and their natural enemies often form a distinct compartment of the larger food web associated with host-plants. In our system, all of the natural enemies are insect parasitoids that complete their development within the gall after parasitizing larva, making it easy to identify and quantify all of the trophic interactions within this food web."

Although reviewer #3 points out that this is still a relatively simple community, we actually feel that our approach of quantifying all of the trophic interactions within a distinct food-web compartment (gall midges and their parasitoids on *Salix hookeriana*) was unique and crucial for understanding the relationship between genetic variation and food-web complexity.

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.

20. As advised, we have omitted our prior claims from the Introduction of the revised manuscript. Specifically, we have omitted those prior claims and combined the former 2nd and 3rd paragraphs of the Introduction into a single paragraph.

Lines 66-83 (2nd paragraph) of the Introduction now reads: "Genetic variation is a key driver of intraspecific variation and many studies have now demonstrated direct and indirect genetic effects on species interactions (8–10) and the composition of communities across multiple trophic levels (11–14). This prior work forms a clear expectation that intraspecific genetic variation is capable of scaling up to affect the structure of an ecological network. In particular, we expect that network structure will be affected by genetic variation through at least two different mechanisms. For a food web (network of trophic interactions), genetic variation in the quality of a basal resource may alter the (i) abundances or (ii) phenotypes of consumer species or both (16). These direct genetic effects on consumers may then have cascading effects on the strength of trophic interactions between consumers and their predators (16), resulting in distinct compositions of trophic interactions associated with different genotypes of the basal resource (Fig. 1). If such genetic specificity in the composition of trophic interactions occurs, then theory predicts that increasing genetic variation will result in more interactions per species (6, 17), and therefore greater food-web complexity (Fig. 2). Moreover, greater complexity may in turn affect food web dynamics, as more complex food webs are predicted to be more robust to species extinctions (1, 18). However, whether genetic variation is capable of scaling up to affect food-web complexity is currently unclear."

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?

21. Figure 1 illustrates that we only documented 12 unique gall-parasitoid interactions within this network. Of course, it is true that the total number of potential interactions in this bipartite network is 24 (i.e. each of the 4 galls could interact with each of the 6 parasitoids, 6*4 = 24). Interspecific differences among gall species (e.g. differences in gall morphology, phenology, plant part galled, etc.) likely constrain the number of potential interactions to considerably less than 24. We are confident though that the 12 unique gall-parasitoid interactions we documented represent the majority of interactions within this food web. To illustrate this, we considered unique gall-parasitoid interactions as 'species' and used Chao 1 to estimate the total number of gall-parasitoid interactions (Gotelli and Colwell 2010, Ch. 4 of Biological Diversity: Frontiers in Measurement and Assessment). Chao 1 estimated there to be 15 unique interactions (std. error = 4.5), suggesting that we have reliably sampled the interactions that contribute most to food-web complexity.

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.

22. Reviewer #3 points out a weakness of our study in that it we only have a single season of data. As a consequence, it is unclear how repeatable our results would be with a 2nd year of study. As a reminder, one of the key findings of this paper was that heritable phenotypic variation shapes network structure. Below, we provide support for this key finding by drawing

upon results from previously published work (Barbour et al. 2015, *Functional Ecology* 29:995-1006), the current manuscript, and results presented at Ecological Society of America's annual meeting in 2015 (ESA poster was published on 'figshare' prior to receiving Reviewer #3's comments on Aug. 27th, http://figshare.com/articles/Food_web_complexity_reduces_variation_in_herbivore_fitness_among_host_plant_genotypes/1525124).

Key finding #1: Barbour et al. (2015) found that leaf C:N was highly heritable ($H^2 = 0.61$) and also was positively associated with the density of leaf galls (*Iteomyia salicisverruca*). Similarly, we found a trend for a positive association between leaf C:N and leaf gall density in the current manuscript.

Key finding #2: In the current manuscript, we found that the density and size of leaf galls varied 10- and 2-fold among willow genotypes, respectively. In support of this finding, work conducted in 2011 (Barbour et al. 2015, *Functional Ecology*) and 2013 (Figure P1, ESA poster) demonstrate that willow genotypes varied 6.7- and 6-fold in leaf gall density, respectively. Moreover, work conducted in 2013 (Figure P1, ESA poster) demonstrates that leaf gall size varied 1.5-fold among willow genotypes.

Key finding #3: We found that the strength of leaf gall-parasitoid interactions varied among willow genotypes. This finding was corroborated by work conducted in 2013 (Figure P2, ESA poster).

Key finding #4: We found that the density and size of leaf galls was an important determinant of the strength of gall-parasitoid interactions. This finding was corroborated by work conducted in 2013 (Figure P3 & P4, ESA poster).

While the previously published work and the study presented in the ESA poster focused on different topics, we feel these results support the key finding of our study that heritable phenotypic variation shapes network structure.

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).

23. We agree with Reviewer #3 that an important limitation of our study was that we did not conduct an experiment that explicitly manipulated genetic variation. However, it is important to note that the qualitative conclusions of our results will still hold unless there are negative, non-additive effects equal or greater in magnitude compared to the additive effect we observed. We discuss these points more clearly in the revised manuscript.

Lines 193-198 now read: "Future experiments are needed that explicitly manipulate levels of genetic variation and test for the presence and magnitude of non-additive effects on food-web structure. It is worth noting though that our qualitative conclusion, namely that genetic variation

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."

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.

24. Reviewer #3 is correct that our experiment is limited to documenting associations between traits and trophic interactions (ref point #4). Therefore, we have toned down our wording to say that we have identified traits that "may be determining" instead of "determine" in the current version of the manuscript.

Lines 239-241 in the *Materials and Methods* now read: "To identify the plant traits that may be determining resistance to galling insects, we measured 40 different traits associated with leaf quality (36 traits) and plant architecture (4 traits)."

We also toned down our causal inferences in Lines 124-131 and 546-547. It was beyond the scope of this work to conduct further experiments to identify the causal traits determining these interactions (e.g. through silencing of genes); therefore, we hope that toning down our inferences is sufficient.

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.

25. As advised by Reviewer #3, we have now included a structural equation model to tease apart the contribution of variation in gall abundances on network complexity. Below, we show how we have incorporated this model into the revised manuscript.

Lines 174-185 of the *Results and Discussion* now read: "To more precisely understand the relationship between genetic variation, the addition of complementary interactions, and food-web complexity, we used a structural equation model (*Materials and Methods*). We found that increasing genetic variation resulted in a more diverse community of galls and a more generalized network of gall-parasitoid interactions, albeit through two main pathways (Fig. S2).

On the one hand, increasing genetic variation resulted in higher gall species richness, which had a positive direct effect on food-web complexity (standardized path effect = 0.38). On the other hand, increasing genetic variation resulted in higher gall abundances, which indirectly increased complexity by increasing the effective number of parasitoid species per gall (standardized path effect = 0.28). Other pathways had comparatively small and idiosyncratic effects on food-web complexity (Fig. S2)."

Lines 369-394 of the *Materials and Methods* now read: "To examine the pathways by which genetic variation influences food-web complexity, we built a piecewise structural equation model (41) using data from one randomly selected replicate of our resampling procedure (of the 40). We observed the same qualitative results when we explored other replicates, so we only report the quantitative results from the first one we selected. For our plant-insect food web, complexity is principally determined by 3 components: (i) the effective number of gall species per willow (i.e. Shannon diversity of galls); (ii) the effective number of parasitoid species per gall (vulnerability, V_q); and (iii) the effective number of gall species per parasitoid (generality, G_q) (38). Increases in any of these 3 components, all else equal, will directly increase food-web complexity. In addition, the total abundance and diversity of galls may indirectly affect complexity by influencing the vulnerability and generality of the gall-parasitoid network. Therefore, we built our structural equation model to incorporate these different pathways. In addition, since species diversity is determined by both the evenness and richness of a community, we partitioned gall diversity into its evenness (E^I = exp(Shannon diversity)/richness) and richness components (40) before building the model. Given the non-linear relationship between genetic variation and food-web complexity (Fig. 6), we restricted our analysis to the first 4-levels of genetic variation. We feel this was justified for two reasons: (i) this was the portion of the relationship that increased the most; and (ii) this was the only portion of the relationship that was mostly linear with constant variance, thereby satisfying the assumptions of linear regression models that made up our structural equation model. Finally, we used a test of directed separation (41), which essentially tests whether there are any significant paths missing from the model. For tests of directed separation, $P > 0.05$ indicates that the model provides a good fit to the data (i.e. no missing paths), whereas $P < 0.05$ indicates a model with missing paths."

Lines 99-107 of the supplementary information now read: "**Structural equation model of food-web complexity** – Fig. S1 shows the data used to evaluate the structural equation model in Fig. S2. We found that this model provided a good fit to the data with no evidence of missing pathways (Fisher $C = 0.88$, $k = 6$, $P = 0.99$). In particular, we found that genetic variation increased food-web complexity primarily by: (i) an increase in gall richness that directly increased complexity (0.49*0.78 = 0.38); and (ii) an increase in gall abundance that indirectly increased complexity by increasing gall vulnerability (0.69*0.62*0.65 = 0.28). Interestingly, gall evenness had a small overall negative effect on complexity ((-0.19*0.58) + (-0.19*-0.32*0.65) + (-0.19*0.28*0.26) = -0.09).