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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 reviewer’s 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 of 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).**

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 have now revised the text in the *Results and Discussion* that precedes this result to highlight this point. Lines \_ - \_ 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.**

From Johnson 2006: Unlike univariate variables, such as plant productivity (e.g. Loreau and Hector, 2001), the expected food-web complexity of diverse patches cannot be easily determined because richness is a composite measure of the number of unique interactions occurring at multiple trophic levels in each patch.

Therefore, it is necessary to use resampling methods to generate expected datasets of genetically diverse patches using the data from the monoculture patches.

3. We agree with Reviewer #1 that the assumptions underlying our computer simulation were not presented clearly enough in the original manuscript. Below, we clarify these assumptions and show where we made the appropriate changes in the manuscript.

(A) Since our quantitative analysis of trophic interactions comes from a common garden mixture of 26 genotypes, our estimates of food-web complexity represent the combined effect of both additive and non-additive processes.

(B) Following on assumption (A), we are assuming that our estimates of food-web complexity for genotype monocultures could substitute for estimates of food-web complexity from an actual experiment. This assumption is valid under two conditions: (1) we sampled sufficiently to estimate food-web complexity for each genotype. (2) non-additive effects are absent from this system. Importantly though, if there are positive, non-additive effects present (e.g. niche partitioning), then our simulation will be *underestimating* the positive relationship between genetic variation and food-web complexity. The inset of Figure 6 suggests that willow genotypes host distinct compositions of trophic interactions. Moreover, we found that gall species were associated with different willow traits, and that the three dominant parasitoids species (*Platygaster*, *Mesopolobus*, and *Torymus*) responded differently to the size and density of the dominant gall species (leaf galler, *Iteomyia salicisverruca*), providing putative mechanisms for why willow genotypes host distinct sets of trophic interactions. Giving these putative mechanisms, it is difficult to imagine negative, non-additive effects, which would suggest we are overestimating the effects of genetic variation on food-web complexity. While we cannot conclude whether this leads to niche partitioning (i.e. species attaining higher abundance) of each of these interactions, they at the very least suggest that

Therefore, while our results do not experimentally examine the consequences of genetic variation for food-web complexity, our results suggest that the positive relationship between genetic diversity and food-web complexity we observed is likely to be both real and an underestimate for our system.

by estimating food-web complexity at different levels of genetic variation, we are assuming that the contribution of additive and non-additive effects in the mixture of 26 genotypes stays the same across all levels of genetic variation. This of course is not true, seeing as how the estimates of food-web complexity for a single genotype should just represent the additive effects. Importantly though, whether violating this assumption qualitatively alters our interpretation (i.e. intraspecific genetic variation increases food-web complexity) depends on whether the non-additive effects in our system are positive, neutral, or negative. Specifically, if non-additive effects are negative, Below, we argue that we argue that this limitation of our data means that we are likely *underestimating* the positive effects of host-plant genetic variation on food-web complexity.

(C) In order for there to be a positive relationship between host-plant genetic variation and food-web complexity, we are assuming that any non-additive effects are either neutral or positive.

If the non-additive effects are neutral, then the positive relationship between food-web complexity and genetic variation would be due entirely to additive effects (i.e. sampling a genotype that has high food-web complexity). If the non-additive effects are positive, then our analysis is overestimating the food-web complexity for single genotype monocultures, which should only represent the additive effects of genotypes.

We have made this more clear in our revised manuscript. Lines \_-\_ now read:

“INSERT LINES”.

Assumptions of simulation:

(1) Non-additive effects of genetic variation on food-web complexity are neutral or positive

**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.

“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, χ23,104 = 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).”

**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. The matrix of microsatellite markers was published in the supplementary material of Barbour et al. (2015, Functional Ecology, Table S1); however, since the willow genotyping was only based on 2 markers, we felt it was unwise to attempt to infer the relatedness of these genotypes.

In terms of the independence of these genotypes, we can examine this by measuring the functional diversity of the 26 genotypes in multivariate trait space. To do this, we calculated the average trait value for each of the 40 traits for each willow genotype. We then calculated functional evenness and functional divergence (FD package in R, Villéger et al. 2008). Values close to zero correspond to functional redundancy, while values close to one indicate functional distinctiveness. We found that functional evenness and functional divergence were equal to 0.94 and 0.87, respectively. These high values of functional evenness and functional divergence suggest that these willow genotypes are mostly independent of each other (in multivariate trait space).

**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, Figure 3A-C and 4A-C in the revised manuscript 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 this because we feel it is important to present the raw data and all of the variability. Indeed, we feel that presenting the raw data allows the reader to have a transparent understanding of our methods and the data we present.

**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. Reviewer #1 is correct in that the dashed line would represent an overestimate of the contribution of additive effects (i.e. sampling) if we had manipulated genetic variation in this experiment. However, as we mentioned in Point #3, we realized that we are unable to estimate the relative contributions of additive and non-additive effects because of our experimental design. Therefore, we have decided to no longer give an “expectation for sampling effects alone” because our data clearly do not permit us to estimate this. We have revised the text accordingly and removed the dashed line from Figure 6. Lines \_-\_ now read:

“INSERT TEXT”.

**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 \_-\_ 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 *H2* = 0.72) and plant architecture (4 traits, mean *H2* = 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-herbivore and herbivore-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 that 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 include details in how we determined the sensitive of our results to the number of simulations as well as the number of plant replicates sampled for each willow genotype. Below, we outline the updated methods. Lines \_-\_ in the *Materials and Methods* now reads:

**“Intraspecific genetic variation increases network complexity.**To examine this, we used our empirical data to simulate the complexity of the plant-insect food web at different levels of genetic variation (range = 1 to 25 genotype polycultures) in the experimental population of willows. We omitted 1 of the 26 genotypes from this analysis (Genotype U) because we never found any galls on the branches we sampled. For each simulation, we performed the following procedure. First, we randomly selected 1 to 25 genotypes. Each genotype selection consisted of a random sample (without replacement) of 4 individual plants and their corresponding trophic interactions (willow-gall and gall-parasitoid). Second, we calculated the average abundance of each trophic interaction, which we then used to calculate food-web complexity. For our index of food-web complexity, we chose to use quantitative-weighted linkage density, *LDq*which is based on Shannon Entropy and is the average of the effective number of prey and predatory interactions for a given species, weighted by their energetic importance (details on how *LDq*was calculated are available in the supplementary information and in 38, 39). *LDq* (hereafter, food-web complexity) is less sensitive to variation in sample size compared to other measures of food-web complexity (39), making it an appropriate measure of complexity for our analysis. We repeated this sampling procedure X times for each level of genetic variation, resulting in Y estimates of food-web complexity over the range of willow genetic variation. We determined that X simulation was sufficient because the estimate slope of this relationship began to reach an asymptote (supplementary information). All statistical analyses were conducted in R (40).”

**- 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 developed throughout the paper.

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.**

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 \_-\_ now read:

“INSECT HERE”

**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. Reviewer #3 brings up a valid point and we should have been clearer in this regard. Indeed, there have been a plethora of studies showing that genetic variation has direct and indirect effects on the composition of pairwise interactions. The main point is that prior work has either aggregated (e.g. combined natural enemies) or ignored (e.g. removing upper trophic levels) members of the community that may be important in determining community dynamics. Our approach was to focus on quantifying interactions within a relatively distinct food-web compartment (gall midges and their parasitoids on *Salix hookeriana*). In doing so, we are quantifying the interactions that are likely of primary importance to community dynamics. We make this distinction more clear in the revised manuscript. Lines \_-\_ now read:

**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 agree with Reviewer #3’s point that the willow-gall-parasitoid community we examined is still a relatively simple one. However, the main reason we focused on this community was because it represents a distinct compartment of the larger food web associated with this species of willow (ref point #18). Much of the prior work on tri-trophic interactions have either lumped all of the natural enemies together or have ignored herbivores that share a similar natural enemy community. Our work suggests that taking a network perspective (i.e. quantifying the direct and indirect effects of host-plant genotype on the composition of all of the possible tri-trophic interactions in a food-web compartment) was critical for understanding the relationship between genetic variation and food-web complexity. Therefore, we disagree with Reviewer #3 that our work represents an ‘incremental’ step. Indeed, we designed our study to collect empirical data on an appropriate scale to (all possible tri-trophic interactions in a food-web compartment) to coincide with food-web theory (e.g. complexity-stability relationship). As mentioned in point #18, we made the following changes to the revised manuscript. Lines \_-\_ now read:

“”

**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.**

We agree with Reviewer #3 one of the main accomplishments of this work is to shown that different genotypes support different interaction networks. Still, what sets our work apart from others is that our interaction network is, to our knowledge, one of the most complete ones quantified because it focused on a small, yet sufficiently diverse, community. This is what we were trying to illustrate with our prior claims, but perhaps at the cost of not de-emphasizing the importance and relevance of this prior work.

**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?**

20. Figure 1 illustrates that we only documented 12 unique gall-parasitoid interactions within this network. It is true of course that the total number of potential interactions in this bipartite network would be 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 represents the vast majority of possible interactions within this food web. To illustrate this, we looked at how the number of unique gall-parasitoid interactions accumulates with the number of willows sampled (supplementary information Figure \_). As you can see, the number of unique gall-parasitoid interactions in this food web begins to saturate near 12, which is the number we documented from our samples.

**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.**

21. 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. Below, we address this concern by drawing upon results from previously published work (Barbour et al. 2015), the current manuscript, and results presented at Ecological Society of America’s annual meeting in 2015 (ESA poster was published online on Aug. 27th at <http://figshare.com/articles/Food_web_complexity_reduces_variation_in_herbivore_fitness_among_host_plant_genotypes/1525124>). While the previously published work and the study presented in the ESA poster focus on different topics, we feel that their results corroborate the point that differences among willow genotypes

Key finding #1: Leaf gall (*Iteomyia salicisverruca*) density and size varied 10- and 2-fold among willow genotypes, respectively. Work conducted in 2011 (Barbour et al. 2015) and 2013 (Figure P1, ESA poster) demonstrate that willow genotypes varied 6.7- and 6-fold in leaf gall density, respectively. Work conducted in 2013 (Figure P1, ESA poster) demonstrates that leaf gall size varied 1.5-fold among willow genotypes.

Key finding #2: Leaf gall survival varies substantially among willow genotypes, primarily due to variation in leaf gall size. In 2013 (ESA poster,

(1) In our study, we found that variation in the density and size of leaf galls (*Iteomyia salicisverruca*) was an important determinant of gall-parasitoid interactions. varied 6.7-fold and 10-fold in density among willow genotypes in 2011 (Barbour et al. 2015, *Functional Ecology*) and 2012 (this manuscript), respectively.

has In particular, Reviewer #3 questions how repeatable our results are and whether the networks would shift. While we cannot fully address this question with our data, we would like to make a number

We disagree with Reviewer #3 in regards to our findings only being applicable if interactions networks remain relatively consistent. We argue that our findings will still hold as long as different genotypes host distinct interaction networks. For example, our results suggest that the positive relationship between genetic variation and food-web complexity we observed was partially due to genotypes hosting different interaction networks. This result would hold in subsequent years as long as genotypes host distinct interaction networks.

For the dominant leaf galler, we have limited data to test this. There is a trend for a positive genetic correlation among genotypes between 2011 and 2012 (square-root transformed data, Pearson’s *r* = 0.33, 95% C.I. = -0.06 to 0.64, t24 = 1.73, *P* = 0.096; 2011 data from Barbour et al. 2015, Functional Ecology). However, there was still differences among genotypes in leaf gall abundance between years. Therefore, genetic variation likely acts as an important source of environmental heterogeneity.

Reviewer #3 does bring up an important issue in that if the interaction networks weren’t consistent between years, then it is definitely more difficult to predict how natural selection will shape network structure. However,

there is still variation in network composition among genotypes. 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).**

22. As mentioned in point #\_, we believe that are conclusion from the simulation is justified because we have provided evidence of niche partitioning, and since there is heritable variation in willow phenotypes that are associated with these niches, increasing intraspecific genetic variation would increase the available ‘niche space’ to be partitioned. Indeed, we noted in the immediately following lines (538-544), that experimental work is necessary to support this conclusion.

Typically, genetic diversity studies have followed species diversity studies. To our knowledge, species diversity studies have not examined the link to food-web complexity, therefore, we argue that this research still does set a high bar.

We do agree though that our the generality of our results need to be corroborated by future experimental work. Lines \_-\_ now read:

“”

**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.**

23. 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 \_-\_ 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).”*

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). We hope that this does not qualify as sufficient cause to reject this manuscript.

**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.**

24. First, we would like to note that we did show that the frequency of gall-parasitoid interactions, at least for the dominant leaf gall, was not solely due to gall abundance. Specifically, our binomial GLMs showed that the probability of gall-parasitoid interactions (i.e. per-capita interaction strength) depended on an interaction between leaf gall size and abundance (Figure 5).

Still, Reviewer #3 brings up an important point. In the supplementary information of the revised manuscript, we have included a structural equation model that examines the contribution of genetic variation, total gall abundance, and frequency of gall-parasitoid interactions to the weighted linkage density metric. Overall, we found that a model including a direct path between genetic variation and food-web complexity provided a significantly better fit than a model where the effect of genetic variation was mediated solely through total gall abundance and frequency of gall-parasitoid interactions. This lends further supports to our argument that the effects of genetic variation on food-web complexity are not simply the result of a sampling effect, but due to different genotypes hosting distinct interaction networks.