

**Department of Zoology**

2370 – 6270 University Boulevard

Vancouver, B.C., Canada V6T 1Z4

Tel: 604-822-2131

Fax: 604-822-6973

www.zoology.ubc.ca

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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 229-231 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 #\_) and we have clarified these assumptions in the revised manuscript.

Assumption #1: Our simulation can only estimate the additive effects of genetic variation on food-web complexity. We clearly state this again on Lines \_-\_:

“”

In our revised simulation, it became clear that we had to account for the fact that estimates of food-web complexity for polycultures are necessarily based off more sampled willow branches than estimates for monocultures. This will bias polycultures toward having higher food-web complexity than monocultures due simply to differences in sampling effort. We can account for this asymmetry in sampling effort though by including the total abundance of galls and gall-parasitoid interactions as covariates when analyzing the relationship between genetic variation and food-web complexity. When we do this, our qualitative conclusion still holds, namely that genetic variation increases network complexity; however, the quantitative effect is lower (\_%, Lines \_-\_). In the revised manuscript, we clearly outline how we accounted for the asymmetry in sampling effort on lines \_-\_:

“”

Although our results are not from an experiment, it is important to consider the conditions under which the qualitative conclusions from our simulation would no longer hold. We make those conditions clear in the revised manuscript. Lines \_-\_ now read:

“An important limitation of this simulation is that it is unable to estimate the contribution of non-additive effects to food-web complexity. Prior work has shown that host-plant genetic variation can have positive (cite), neutral, or negative (cite) non-additive effects on pairwise species interactions that then influences the diversity of upper trophic levels (27, 28). 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 complexity. However, it is worth noting that the qualitative conclusion of this simulation will still hold unless negative, non-additive effects are equal or greater in magnitude than the additive effects we observed.

**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 184-191 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, 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. 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. In the revised manuscript, we address this in two ways: (1) we notify the reader that the relatedness among genotypes is unknown; and (2) we calculated the functional diversity (evenness and divergence) of the 26 willow genotypes in multivariate trait space to examine the degree of phenotypic redundancy among genotypes. Our analysis of functional trait diversity suggests that there is little phenotypic redundancy among genotypes, suggesting that the unknown relatedness of willows likely has little consequences on our results.

Lines 250-253 of the main text now read, “While relatedness among these genotypes is unknown, analysis of functional-trait evenness and divergence suggests there is little redundancy in the phenotypes (i.e. non-independence) among these genotypes (details in supplementary information).”

Lines 24-31 of the supplementary material now read, **“Relatedness and phenotypic redundancy of willow genotypes –** The matrix of microsatellite markers for the 26 willow genotypes used in this study was published in Table S1 of Barbour et al. (2015); however, since the willow genotyping was only based on 2 markers, they were unable to infer the relatedness of these genotypes. If certain genotypes are more closely related to each other, and consequently have similar phenotypes, this could introduce spurious confidence in our associations between willow traits and gall abundances. We can examine the degree of phenotypic redundancy among the 26 genotypes by measuring their functional evenness and divergence in multivariate trait space (Villéger et al. 2008). To do this, we calculated the average 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 there is little phenotypic redundancy among these willow genotypes. 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, 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. 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 *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 241-243 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 110-116 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 into the following paragraph (Lines 66-83):

“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 vast majority of possible interactions within this food web. To illustrate this, we examined how the number of unique gall-parasitoid interactions accumulates with the number of willows sampled using the method of Colwell et al. (2004, *Ecology* 85:2717-2727). Figure S1 of the supplementary information shows how the number of unique gall-parasitoid interactions in this food web begins to saturate near 12, which is the number of interactions 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.**

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*), the current manuscript, and results presented at Ecological Society of America’s annual meeting in 2015 (ESA poster was published on ‘figshare’ 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 (*H2* = 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 diversity. In the revised manuscript, we make it clear that future experiments are needed to corroborate our result that genetic diversity increases food-web complexity. Lines 242-272 now read:

“An important limitation of this simulation is that it is unable to estimate the contribution of non-additive effects to food-web complexity. It is worth noting though that the qualitative conclusion of this simulation will still hold unless negative, non-additive effects are equal or greater in magnitude than the additive effects we observed. Prior work has shown that host-plant genetic variation can have positive (cite), neutral (cite), or negative (cite) non-additive effects on pairwise species interactions that then influences the diversity of upper trophic levels (27, 28). 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 complexity.”

**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 319-321 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.**

25. 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. Indeed, it became clear from our modified simulation (to address point #\_) that we need to account for sampling effort (e.g. number of galls and gall-parasitoid interactions). However, in this context total gall abundance and the frequency of gall-parasitoid interactions would account for both a nuisance variable (i.e. sampling effort) as well as the potential effect of selecting willow genotypes that had more galls, and as a consequence, more complex food-webs. Therefore, by accounting for gall abundance and frequency of gall-parasitoid interactions as covariates, we can isolate the contribution of willow genotypes hosting distinct and complementary trophic interactions in the contribution to food-web complexity.

Since total gall abundance is now a nuisance variable, we felt it was unwise to conduct a structural equation model to tease apart the direct and indirect effects of genetic variation on food-web complexity. The reason for this is because the indirect effects will include both real effects (i.e. sampling genotypes with high complexity) as well as artifacts of the simulation (i.e. sampling more plants will sample more galls). Therefore, we just included total gall abundance as a covariate in our analyses.