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

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Abstract

Theory predicts that intraspecific genetic variation can increase the complexity of an ecological network. To date though, we are lacking empirical knowledge of the extent to which genetic variation determines the assembly of ecological networks, as well as how the gain or loss of genetic variation will affect network structure. To address this knowledge gap, we used a common garden experiment to quantify the extent to which heritable trait variation in a host plant determines the assembly of its associated insect food web (network of trophic interactions) and drives overall food-web complexity. We found that trait variation among host-plant genotypes **was associated with** resistance to insect herbivores, which in turn indirectly affected interactions between herbivores and their insect parasitoids. Direct and indirect genetic effects resulted in distinct compositions of trophic interactions associated with each host-plant genotype. Moreover, we found that food-web complexity increased by 50% over the range of genetic variation in the experimental population of host plants. Taken together, our results indicate that intraspecific genetic variation can play a key role in structuring ecological networks, which may in turn affect network persistence.

Significance

We know that the gain or loss of species can have cascading effects on the complexity of a food web; however, it is less clear whether the gain or loss of genetic variation within species, an often over-looked component of biodiversity, will similarly affect food-web structure. Here, we identify how genetic variation within a host plant directly and indirectly affects its associated insect food web, resulting in distinct trophic interactions occurring on each host-plant genotype. Moreover, we found that higher levels of host-plant genetic variation lead to a more complex plant-insect food web. Our results suggest that preserving genetic variation within key species may be critical for maintaining complex and robust food webs under future environmental change.

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Introduction

Network theory has provided both a conceptual and quantitative approach for mapping interactions between species and making predictions about how the gain or loss of species will affect the structure and dynamics of ecological networks (1–3). Representing a network at the species-level, however, makes the implicit assumption that each species consists of a homogenous population of individuals, all of which interact equally with individuals of different species. Yet, most populations are heterogeneous mixtures of individuals that vary in their phenotypes and there is growing evidence that this intraspecific variation is an important factor governing the assembly of ecological communities (4–6). Consequently, there is a clear need to account for the role of intraspecific variation in structuring ecological networks (7).

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.**

In this study, we quantify the genetic specificity of trophic interactions and **examine whether** increasing genetic variation results in greater network complexity using a common garden experiment of a host plant (26 genotypes of coastal willow, *Salix hookeriana*) and its associated food web of insect galls and parasitoids (Fig. 1). We focused on this plant-insect food web for three reasons. 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). 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**. Third, the biology of galls is also ideal for identifying the mechanisms mediating trophic interactions. In particular, gall size is a key trait that affects the ability of parasitoids to successfully oviposit through the gall wall and into the larva within the gall (i.e. larger galls provide a refuge from parasitism, 21). Moreover, gall size is determined, in part, by the genotype of the plant (21), so we have a clear mechanism by which genetic variation can affect the strength of trophic interactions. Taken together, our study seeks to test theoretical predictions for how intraspecific genetic variation influences the structure of ecological networks. In doing so, our study takes a crucial step toward a more predictive understanding of how the gain or loss of genetic variation will affect the dynamics of ecological networks.

Results and Discussion

Quantifying the genetic specificity of the plant-insect food web. In concordance with previous work in this system (19), we observed clear differences in the abundance of 3 of the 4 galling insects among willow genotypes (multivariate GLM, $\chi^2_{25,119} = 202.40$, $P = 0.001$; Table S1). Specifically, we found that the average abundance of leaf, bud, and apical-stem galls varied 10-, 8-, and 1.4-fold among willow genotypes, respectively (Fig.

3A-C). This variation resulted in 69% dissimilarity in the average composition of gall communities among willow genotypes ($F_{22,89} = 1.96$, $P = 0.001$). Moreover, we found that the average diameter of leaf galls varied 2-fold among willow genotypes (Fig. 3D). This observed genetic specificity in the abundance and phenotypes of insect herbivores corroborates decades of work in other plant-gall (8, 11, 21) and plant-herbivore systems (12, 15).

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). These results highlight that accounting for intraspecific variation in multiple plant traits is important for predicting antagonistic interactions between plants and insect herbivores (19), and should therefore be incorporated into mechanistic models of food-web structure.

We found that the effects of willow genetic variation extended beyond pairwise interactions with herbivores (11, 12, 15) and simple tri-trophic interactions (8–10, 21) to determine the assembly of the network of gall-parasitoid interactions (multivariate GLM, $\chi^2_{25,119} = 357.10$, $P = 0.001$; Table S1). In particular, we found that the frequency of parasitism from three parasitoids (*Platygaster* sp., *Mesopolobus* sp., and *Torymus* sp.) on leaf galls varied 270%, 30%, and 40% among willow genotypes, respectively (Fig. 4A-C). This variation resulted in 78% dissimilarity in the average composition of gall-parasitoid interactions among willow genotypes ($F_{12,45} = 1.57$, $P = 0.007$). Furthermore, we found that the probability of a gall being parasitized also depended on willow genotype (Table S1), a pattern that was particularly strong for leaf galls (Fig. 4D).

The genetic specificity of the network of gall-parasitoid interactions was determined by variation in both the abundance and size of galling insects. Specifically, we found that the abundance of 67% (8 of 12) of the gall-parasitoid interactions increased with the abundance of their associated galls, and that leaf gall size affected trophic interactions with both leaf and bud galls (multivariate GLM, $\chi^2_{4,76} = 179.80$, $P = 0.001$; Table S2). In terms of interaction strength, we found that the odds of a leaf gall being parasitized decreased by 25% with every 1 mm increase in leaf gall diameter (GLM, $\chi^2_{1,79} = 22.28$, $P < 0.001$). Nevertheless, the strength of trophic interactions with individual parasitoid species depended on both leaf gall size and abundance (Fig. 5A-B; Table S3), suggesting that natural selection has the potential to shape food-web structure. For example, if there were selection on willows for increased resistance to leaf galls through smaller galls and lower gall abundances, then we would expect to see more parasitism overall and a shift in dominance from *Platygaster* to *Mesopolobus*, since *Mesopolobus* had its highest attack rates on small galls at low abundances (Fig. 5A). While our results are limited to examining the effects of standing genetic variation on a tri-trophic food web over a single season, there is ample evidence from other studies that natural selection can play an important role in shaping consumer-resource dynamics (22, 23). Understanding how evolutionary processes affect the structure and dynamics of ecological networks, and vice versa (24, 25), is likely a fruitful topic for future research.

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*). **After accounting for sampling effort (dashed line, Fig. 6), we found that food-web complexity increased by 20% with increasing genetic variation (Fig. 6). This positive relationship was primarily due to an increased likelihood of sampling genotypes with complementary trophic interactions, as we found that** willow genotypes differed by 73% in the average composition of their trophic interactions **(inset Fig. 6). 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).**

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.**

Conclusions

Our results suggest that the gain or loss of genetic variation within a key species can fundamentally alter food-web complexity and therefore the persistence of food webs. There are two main conclusions from our work. First, intraspecific variation in multiple traits is an important driver of network structure; therefore, mechanistic models of food-web structure should incorporate such variability within species (7), as this can enhance the accuracy of these models in predicting trophic interactions (29). Given that plants, insect herbivores, and their parasitoids comprise over half of all known species of metazoans (30, 31), accounting for intraspecific variation in a wide range of functional traits should be a priority for future food web models (32). Second, understanding the direct and indirect effects of genetic variation on trophic interactions is essential for predicting how evolutionary processes will affect the structure and persistence of food webs over time. Indeed, our analysis suggests that the loss of genetic variation will result in less complex food webs. Moreover, genetic variation provides the raw material for evolution by natural selection; therefore, losing genetic variation in key species may hinder the adaptive capacity of both the species and the food web under future environmental change (33, 34). **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.** Given that the current rate of population extinction is orders of magnitude higher than the rate of species extinction (35), our study highlights the pressing need for research examining how the loss of genetic variation within and among populations will affect food webs and the ecosystem services they provide (36, 37).

Materials & Methods

Common garden experiment and plant traits. To isolate the effects of coastal willow (*S. hookeriana*) genetic variation on the plant-insect food web, we used a common garden experiment consisting of 26 different willow genotypes (13 males; 13 females), located at Humboldt Bay National Wildlife Refuge (HBNWR) (40°40'53"N, 124°12'4"W) near Loleta, California, USA. Willow genotypes were collected from a single population of willows growing around Humboldt Bay. **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. This common garden was planted in February 2009 with 25 clonal replicates (i.e. stem cuttings) of each willow genotype in a completely randomized design in two hectares of a former cattle pasture at HBNWR. Willows in our garden begin flowering in February and reach their peak growth in early August. During this study, willows had reached 2 - 4 m in height. Further details on the genotyping and planting of the common garden are available in (19).

To identify the plant traits that **may be** determin**ing** resistance to galling insects, we measured 40 different traits associated with leaf quality (36 traits) and plant architecture (4 traits). Each of these 40 traits exhibited significant, broad-sense heritable variation (mean leaf quality $H^2 = 0.72$; mean architecture $H^2 = 0.27$; range of H^2 for all traits = 0.15 - 0.97). For further details on how these willow traits were sampled and quantified, see methods in (19). We then reduced these 40 traits into 13 composite traits that had a negligible degree of multicollinearity using either principle components analysis (PCA), sequential regression (residuals of one trait after accounting for correlation between two traits), or removing one trait from a pair of highly correlated traits (details on methods in 19). The final set of leaf quality traits included salicylates/tannins PC1, flavones/flavonols PC1-2, phenolic acids PC1-2, flavanones/flavanonols PC1 (Table S3 of 19), carbon-to-nitrogen ratio (C:N), water content, specific leaf area (residuals from water content), and trichome density. The final set of plant architecture traits included plant size, plant height (residuals from plant size), and foliage density (residuals from plant size).

Quantifying the genetic specificity of the plant-insect food web. To build a quantitative food web for each willow genotype, we collected galls from about 5 randomly chosen replicates of each genotype in September 2012 (N = 145 willows, range = 4 - 9 replicates per genotype). For each replicate willow, we collected all galls occurring on one randomly selected basal branch. We restricted our gall collections to those induced by midges in the insect family Cecidomyiidae. These species included a leaf gall (*Iteomyia salicisverruca*), bud gall (*Rabdophaga salicisbrassicoides*), apical-stem gall (unknown midge species), and mid-stem gall (*Rabdophaga salicisbattatus*). To quantify the abundance of gall-parasitoid interactions, we placed collected galls into 30 mL plastic transport vials (loosely capped at the end), which we maintained at room temperature in the lab for four months. We then opened galls under a dissecting scope and determined whether the gall survived or was parasitized, and if parasitized, the identity of the parasitoid species. In total, we identified five species of hymenopteran parasitoids, including *Platygaster* sp. (Family: Platygastridae), *Mesopolobus* sp. (Family: Pteromalidae), *Torymus* sp. (Family: Torymidae), *Tetrastichus* sp. (Family: Eulophidae), and an unknown species of Mymaridae (hereafter, Mymarid sp. A), as well as one predatory midge (*Lestodiplosis* sp., Family: Cecidomyiidae). This predatory midge is functionally similar to the other parasitoids so we collectively referred to this natural enemy community as parasitoids for brevity. We omitted from analyses those galls for which we could not reliably determine the cause of mortality. We quantified gall abundance by counting the number of surviving and parasitized larva for each gall species collected from each branch. For gall size, we measured galls to the nearest 0.01 mm at their maximum diameter (perpendicular to the direction of plant tissue growth).

To quantify the genetic specificity of trophic interactions with galling insects, we tested for differences in gall sizes, abundances, and community composition among willow genotypes. For gall size, we analyzed separate linear models with willow genotype as the predictor variable and average gall size as the response variable, but we weighted the analysis by the number of galls used to calculate average gall size. We weighted the analysis because we expected that averages based on more galls reflect a more accurate estimate of the average size of galls found on a willow individual. For gall abundances, we analyzed multivariate generalized linear models (multivariate GLMs, error distribution = negative binomial, link function = log) with willow genotype as the predictor variable and a matrix of gall abundances as the response variable. For gall community composition, we used permutational MANOVA (PERMANOVA) with willow genotype as the predictor variable and a matrix of Bray-Curtis dissimilarities in gall abundances as the response variable. To identify the plant traits mediating resistance to galling insects, we used the same analyses as for gall sizes (weighted linear models) and abundances (multivariate GLMs) except that our predictor variable was now a matrix of willow traits. To select a final model of willow traits, we sequentially removed traits based on Aikaike information criteria (AIC) to identify a nested set of candidate statistical models. We then used likelihood ratio tests to identify the statistical model of willow traits that best predicted gall abundances or gall sizes.

To quantify the genetic specificity of the network of gall-parasitoid interactions, we tested for differences in the abundance, composition, and strength of gall-parasitoid interactions among willow genotypes. For the abundance and composition of gall-parasitoid interactions, we used the same analytical approach as we did to test for differences in gall abundances and community composition. For these analyses though, we had a matrix of the abundance (multivariate GLMs) or dissimilarity (PERMANOVA) of unique gall-parasitoid interactions as the response variable. To identify the mechanisms determining the abundance of gall-parasitoid interactions, we again used multivariate GLMs except that our predictor variable was now a matrix of gall abundances and gall sizes. We then used the same approach as we did to identify the willow traits that best predicted gall abundances (i.e. AIC and likelihood ratio tests), to identify which gall sizes and abundances best predicted the abundance of gall-parasitoid interactions. For the strength of gall-parasitoid interactions, we used separate GLMs (error distribution = binomial, link function = logit) with willow genotype as the predictor variable and the proportion of galls parasitized as our response variable for each gall species. If we detected an effect of willow genotype on total parasitism rates, then we analyzed separate GLMs for each parasitoid species to determine which parasitoids were driving total parasitism rates. Finally, we again used AIC and likelihood ratio tests to examine whether parasitism rates were due to gall abundance, gall size, or their interaction.

Intraspecific genetic variation increases network complexity. For our index of complexity, we chose to use quantitative-weighted linkage density, LD_q, which is based on Shannon diversity 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 LD_q was calculated are available in the supplementary information and in 38, 39). LD_q (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 study.

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. 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.

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.

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^1 = 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.

All statistical analyses were conducted in R **version 3.1.2** (40).

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Figure Legends

Fig. 1. Genetic specificity of trophic interactions in a plant-insect food web. This food web represents the trophic interactions aggregated from all plant individuals sampled in this common garden experiment, whereas each genotype subweb represents the trophic interactions aggregated from all plant individuals of the corresponding genotype. We depicted three genotype subwebs (of 26) to illustrate the differences in trophic interactions associated with each willow genotype. The species comprising this food web include a host plant (coastal willow, *Salix hookeriana*), four herbivorous galling insects, and six insect parasitoids (species details in *Materials & Methods*). The width of each grey segment is proportional to the number of individuals associated with each trophic interaction. Note that we scaled the width of trophic interactions to be comparable among genotype subwebs, but not between subwebs and the aggregated food web, in order to emphasize the differences among subwebs.

Fig. 2. Conceptual model of how increasing genetic variation (number of shades of green circles) results in greater food-web complexity (number of interactions per species). If different genotypes of a basal resource are associated with distinct compositions of trophic interactions (i.e. genetic specificity of trophic interactions), then increasing genetic variation in the resource will result in a more complex food web because of the increase in the number of interactions per species at all three trophic levels. Colors correspond to different trophic levels (green = basal resource, blue = primary consumer, orange = secondary consumer), while different shapes within each trophic level correspond to different species.

Fig. 3. Direct effects of willow (*Salix hookeriana*) genetic variation on its associated community of galling insects. Among the 26 willow genotypes we surveyed in our common garden experiment, we found that: (A) average abundance of leaf galls varied 10-fold (GLM, $\chi^2_{25,119} = 74.60$, $P = 0.001$); (B) average abundance of bud galls varied 8-fold (GLM, $\chi^2_{25,119} = 55.02$, $P = 0.006$); (C) average abundance of apical-stem galls varied 1.4-fold (GLM, $\chi^2_{25,119} = 44.47$, $P = 0.042$); and (D) average diameter of leaf galls varied 2-fold (weighted linear model, $F_{23,57} = 2.17$, $P = 0.009$). Plots (*A* – *C*) display the median (bar within box), 25th to 75th percentiles (IQR, box edges), 1.5 × IQR (whiskers), and outliers (points) for gall abundances found on each willow genotype. For plot (*D*), each circle corresponds to the average gall diameter associated with an individual willow and the size of the circle is scaled according to the number of galls used to calculate the weighted average for each willow genotype (diamond). Colors correspond to different gall species (orange = leaf gall, blue = bud gall, grey = apical-stem gall). For all plots, we ordered willow genotypes based on average leaf gall abundance (low to high).

Fig. 4. Indirect effects of willow (*Salix hookeriana*) genetic variation on its associated network of gall-parasitoid interactions. Among the 26 willow genotypes we surveyed in our common garden experiment, we found that: (*A*) leaf gall parasitism by *Platygaster* sp. varied 270% (GLM, $\chi^2_{25,119} = 79.51$, $P = 0.001$); (*B*) leaf gall parasitism by *Mesopolobus* sp. varied 30% (GLM, $\chi^2_{25,119} = 50.00$, $P = 0.009$); (*C*) leaf gall parasitism by *Torymus* sp. varied 40% (GLM, $\chi^2_{25,119} = 60.11$, $P = 0.001$); and (*D*) the proportion of leaf galls

parasitized varied between 0.0 and 1.0 (GLM, $\chi^2_{23,58} = 75.79$, $P < 0.001$). Plots (*A* – *C*) display the median (bar within box), 25th to 75th percentiles (IQR, box edges), 1.5 × IQR (whiskers), and outliers (points) for the abundance of gall-parasitoid interactions associated with each willow genotype. For plot (*D*), each circle corresponds to the proportion of galls parasitized on each replicate willow and the size of the circle is scaled according to the number of galls used to calculate the weighted average for each willow genotype (diamond). Colors correspond to different gall-parasitoid interactions. As with Fig. 3, we ordered willow genotypes based on average leaf gall abundance (low to high).

Fig. 5. Variation in the size and abundance of leaf galls on willows **is associated with changes in** the strength and composition of gall-parasitoid interactions. (*A* – *B*) In general, the proportion of leaf galls parasitized by both *Platygaster* (blue, solid line) and *Mesopolobus* (green, short-dashed line) decreases as gall size increases, while *Torymus* (orange, long-dashed line) exhibits the opposite pattern. On willows with small leaf galls though (< 8 mm), *Mesopolobus* had the highest attack rate at low gall abundances (1 – 4 leaf galls per branch, N = 46 per parasitoid species), whereas *Platygaster* was the dominant parasitoid at high gall abundances (5 – 22 leaf galls per branch, N = 35 per parasitoid species). Lines correspond to slopes estimated from generalized linear models (GLMs). Points were jittered slightly to avoid overlapping values.

Fig. 6. Increasing willow (*Salix hookeriana*) genetic variation results in a more complex plant-insect food web due to complementarity in trophic interactions. Specifically, we found that the average complexity (LD_q, quantitative-weighted linkage density) of the

plant-insect food web increased by **2**0% over the range of genetic variation (number of genotypes) in the experimental population of willows. Grey circles correspond to **the average** food-web complexity estimates for **each replicate simulation** (N = **40 for each level of genetic variation)**, whereas blue circles correspond to the **overall** average complexity of food webs at each level of genetic variation. **Black circles correspond to the average complexity of monocultures at 4 different levels of sampling effort (i.e. plants sampled), and the dashed line represents the predicted increase in monoculture complexity with greater sampling effort.** The inset shows how the average composition of trophic interactions (willow-gall and gall-parasitoid) differed by 73% among willow genotypes (PERMANOVA on Bray-Curtis dissimilarities, $F_{22,89}$ = 1.90, P = 0.001), suggesting an important role of complementarity in determining food-web complexity. In this ordination plot, black letters and grey ovals correspond to the centroid and standard error of the centroid, respectively, for the composition of trophic interactions found on each willow genotype. Centroids and their standard errors were calculated from a constrained analysis of principal coordinates (CAP) on Bray-Curtis dissimilarities.