**Title: Interacting foundation species hypothesis: how genetics-based interactions affect community diversity, stability and network structure.**

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**Abstract:**

We examined the hypothesis that genetic-based interactions between foundation species plays an important role in defining community diversity, stability, and species interaction networks for which little is known, but central to understanding community structure and evolution. In a two year experimental manipulation of the tree (*Populus angustifolia*) and its aphid herbivore (*Pemphigus betae*), we found that the interactions of these two foundation species defined: 1. the composition of an arthropod community of 149 species, 2. arthropod community diversity, 3. the stability of arthropod communities through time, and 4. the structure of species interaction networks. These findings represent an important step in evaluating evolution in a community context and they suggest that perturbations that affect the interactions of foundation species are likely to create destabilizing conditions on their associated communities.

**Introduction:**

Understanding the factors that contribute to the structure and stability of communities has a long history in ecological theory (Elton 1947 : MacArthur 19?? other refs). While having often been viewed as an emergent property due to its seemingly endless complexity, new research shows that the intraspecific genetic variation in the multivariate traits of plants (Holeski *et al.* 2012) can define a diverse community of organisms from microbes to vertebrates (review by Whitham et al. 2012), and that the genetics-based interactions of a few strongly interacting species can define a much larger community (Bailey and Whitham 2002; Mooney and Agrawal 2008; Keith *et al*. 2010; Busby *et al*. 2014; other refs?). These genotypic effects can be so consistent and long-lasting that individual herbivores can become locally adapted to individual plant genotypes (Mopper 1996; Evans *et al*. 2008, 2012). Furthermore, community stability or the tendency for species composition to stay the same over time has been found to be, in part, based upon the underlying genetics of individual plant genotypes (Keith *et al*. 2010). Because climate change is an agent of selection acting upon plant populations (Franks *et al*. 2006, O’Neill *et al*. 2008, Sthultz *et al*. 2009) and the communities they support (Gehring *et al*. 2014), it is increasingly important to understand the mechanisms and drivers of community stability.

It is widely accepted that individual species differ in their interaction strengths and their influence on other community members. Foundation species represent the epitome of strong interactors and they are defined as those that structure a community by creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes (Dayton, 1972). In their review, Ellison *et al*. (2005) argued that foundation species are synonymous with keystone species, ecosystem engineers, dominant species and other similar terms (Grime 1984, Hollings 1992, Jones *et al*. 1994, Houston *et al*. 1994). Importantly, all of these terms refer to species of large effect, such that their loss from an ecosystem would have significant effects on other species and ecosystem processes. While many studies have demonstrated the effects of foundation species on their associated communities and ecosystem processes, this fundamental concept raises many key issues of ecological, evolutionary and conservation importance. For example, how many foundation species does an ecosystem have, how do the interactions of multiple foundation species affect community diversity and stability, and are the interactions of foundation species genetically-based such that whole communities may ecologically and evolutionarily change in response to the evolution occurring among interacting foundation species?

To address these questions, it is critical to understand how genetic variation in foundation species affects other community members and how genetics-based interactions of multiple foundation species can potentially drive an associated community. Keith et al. (2010) proposed the Interacting Foundation Species (IFS) Hypothesis in which the genetics-based interactions of two or more foundation species defined a much larger community. Although these studies lacked the critical experiments to confirm or falsify this hypothesis, here, we present findings from common garden experiments designed to address this hypothesis and discuss their general implications.

Cottonwoods are foundation riparian tree species in the American west (Eckenwalder1997, Whitham et al. 2010) that are the focus of much conservation due to climate change and loss of habitat to invasive exotics (Friedman et al. 2005, Grady et al. 2011) and the large number of species that are dependent upon them for their survival (e.g., Ferrier et al. 2011, Bangert et al. 2013).

To test the IFS Hypothesis, we used resistant and susceptible replicates of 10 different individual tree genotypes in a common garden. We experimentally removed aphid and examined several arthropod community traits such as arthropod abundance, species richness and Shannon’s diversity index across a two year period. We also examined the impact of *P. betae* on community stability and interaction network structure. We predicted that if community diversity and stability were related to tree genotype and susceptibility to the aphid (Keith 2010), then the network structure of interacting species would also respond to our treatment. In other words the exclusion of a foundation herbivore would not only affect community composition, richness, and abundance, but also community interaction network structure and the turn-over of species between years (i.e. stability). These complex community traits would therefore be genotype dependent, subject to natural selection, and potentially important to conservation biologists. Our results show that tree genotype and community richness can be used as predictors of arthropod community stability across years and community network structure is influenced by the genetically based interaction between narrowleaf cottonwood and *P. betae*. These findings confirmed our hypotheses that genetic based interactions of foundation species can define complex properties of associated communities.

**Materials and Methods:**

***Experimental Garden Studies:*** Because previous studies had shown differences in community composition and stability in aphid resistant and susceptible tree genotypes, we designed an aphid exclusion experiment using replicated tree genotypes in a common-garden. Common-gardens allowed controlling for plant genotype while also minimizing environmental variation. The common gardens used are 16-yrs-old and located at the Ogden Nature Center in Ogden, Utah. The gardens contain replicated genotypes of *Populus angustifolia* trees collected from along the nearby Weber River. Trees were planted in a random arrangement to minimize the possibility for spatial-autocorrelation. This cottonwood is a dominant of riparian habitats and is widely distributed throughout the Rocky Mountains of the United States and southern Canada (Eckenwalder 1984).

***Aphid exclusion treatment:*** The lifecycle of *Pemphigus betae* allowed for a simple method to intercept gall creating aphid stem mothers en route during their spring migration from the base of the tree up toward awaiting leaf buds. Tangle-Trap insect trap coating was used to exclude aphid stem mothers on treatment branches. An approximately 1/2” thick by 1” wide coating was applied around the base of each treatment branch to prevent stem mothers from reaching sites for gall initiation. Very few aphids are able to circumvent this method of prevention so treatment effect is usually high (**Fig 1**).

***Arthropod Sampling:*** Arthropods were visually censused using the methods of Keith et al. (2010). Equal diameter branches were selected to standardize for leaf area (Fischer et al. 2004) and chosen to be in a north/south opposition. Control and exclusion (treatment) branches were surveyed at similar times on different days to lessen effects of disturbance. Unknown arthropods were collected, later identified to species or morphospecies within a family or genus, and placed in a reference collection (see Appendix A). The community was sampled once per year at a time when previous studies had shown that community diversity was at its greatest (Wimp et al. 2004, 2005). We performed all surveys within a 2 week period (June 7th-21st) under similar weather conditions and times of day. Trees were visually censused for 20 minutes or until all branches had been surveyed (Sensu Wimp 2003). Trees within the garden were surveyed randomly to minimize any potential time-of-day effects. Because plant ontogeny affects arthropod communities in this system (Holeski et al. 2009), we standardized developmental factors by surveying branches only in the non-flowering, juvenile portion of each tree.

***Network Modeling and Analysis***

We used a network approach to examine the impact of *P. betae* removal on the complex set of interactions in the cottonwood-arthropod community. To do this, we constructed tree genotype by arthropod species adjacency matrices using the observations from 2009 (i.e. the second year of the removal treatment). As previous work has demonstrated that plant-herbivore networks tend to form modules (groups of species that tend to interact more strongly with each), which contribute to stability in these communities (Thébault and Fontaine 2010), we focused our analyses on modularity. We calculated modularity using the metric developed by Newman (2004), using a method developed for bipartite or two-mode networks (Dormann and Strauß 2013). To test for the significance of the observed modularity, we compared the observed modularity for both the control and removal networks to the modularity of simulated communities using a standard null modeling approach (Gotelli 2000). We chose to use the most conservative null model, which preserved the marginal frequencies (i.e. the total number of arthropod species on each tree as well as the total abundance of each arthropod across all trees) (Dormann, Gruber and Fruend 2008). We also calculated the contribution of *P. betae* to the structure of each network using the null vector of *P. betae* from the simulated communities (Saavedra et al. 2011). We used a total of 5000 null communities using a sequential swap algorithm with a 100-simulation burn-in. We calculated standardized statistics (z-values) and one-tailed, directional p-values using the modularity of the null communities to test for whether or not the observed modularity or contribution to modularity was greater than the simulated communities.

***Statistical Analyses:***

We used a Generalized Linear Model (GLM) with genotype, herbivore abundance, and genotype x herbivore abundance (e.g. GxE-biotic) as fixed factor effects to compare arthropod abundance and species richness on treatment versus control trees. Data was normally distributed and required no tranformations. Shannon’s (H’) was used to assess arthropod community diversity. Statistical packages used for these analyses were JMP 10 pro and PCORD5). All network modeling and analyses were conducted in R version 3.1.0 (R Development Core Team 2014).

**Results:**

***Experimental aphid removal*** - Colonizing aphid stem mothers were successfully prevented from reaching selected survey branches to form a gall (**Fig. 1**). Because treatment and control branches were on the same tree genotypes, but which differed in their resistance to these aphids, we could critically separate the effects of aphids on community traits, the effect of individual tree genotypes and their interactions, which no previous studies had accomplished as they lacked this experimental design.

Consistent with the hypothesis that galling aphids are a foundation species or strong interactor in their own right, using a generalized linear model (GLM) we found that control branches on susceptible trees (i.e. with galls) supported up to 40% greater arthropod abundance (F15, 112=3.97, *P* <0.0001) and species richness (F15,112=3.04, *P* = 0.0004) than treatment branches without galls.

Because tree genotype alone has already been shown to affect arthropod abundance and richness we also explored this alternative hypothesis. On all susceptible tree genotypes the presence of the aphid was a stronger predictor for arthropod abundance than genotype (F1,7=7.40, *P*=0.007). However, when we examined arthropod species richness across all trees, both genotype and treatment showed significant effects indicating that tree genotype and the presence of the aphid contributed to differences F1,1=5.0, *P*=0.0276) (F1,7=6.0, *P*<0.0001) with genotype being a slightly stronger predictor respectively (**Fig. 2a,b)**. Using only aphid susceptible trees linear regression of tree genotype on residual richness was not significant and suggested the genotype effect on species richness may be better explained by the increase in total arthropod abundance due to not just the presence of the aphid but its own abundance (i.e. degree of susceptibility). When we then performed regression of aphid abundance on species richness we found a significant effect (P=0.0219) indicating that species richness was determined by aphid abundance. This result confirms our hypothesis that it is the interaction of the two species (aphid presence and plant genotype) that significantly affects arthropod abundance and richness which then leads to greater community diversity and stability.

Using Shannon’s diversity index (H’) in our GLM we found results similar to abundance and richness; when all genotypes were included differences among treatment and control were significant (F19,136=2.08, *P*=0.005) with genotype being the significant effect. However when examining only susceptible genotypes, both treatment and genotype were significant. This again supports our hypothesis that both plant genotype and presence of the herbivore interact to determine arthropod community diversity.

Also consistent with the hypothesis that community stability is dependent on both species interactions and plant genetic factors, we used differences in Bray-Curtis similarity to estimate the effects of the two species interaction on the stability of the arthropod community for each individual tree genotype across two years. We found that the presence of the aphid increased the average stability of the community across years with susceptible tree genotypes experiencing greater effects on stability due to treatment. While resistant genotypes showed little or no treatment effect and consistently resulted in a less stable community. (**Fig. 3).** We then calculated an herbivore effect size using Cohen’s D (D=1.16, *ryi* = 0.50) which suggests there is a non-overlap of approximately 33% in the arthropod communities between treatment and control (i.e. with and without the aphid).

*Pemphigus betae* removal altered the cottonwood-arthropod interaction network structure. Modularity was significantly different from the null communities for the exclusion (modularity = 0.2, p = 0.001), but not for the control network (modularity = 0.17, p = 0.91). The contribution of *P. betae* to the modularity was low in the exclusion treatment (z = -0.13), and in the control P. betae tended to reduce modularity (z = -0.65), indicating a suppression of module formation. Tree genotype contribution to modularity was positively correlated with the effect of *P. betae* removal (i.e., control PB – exclusion PB) showing a significant positive correlation (Pearson’s r = 0.33, p = 0.037).

**Discussion:**

**Genetics-based IFS**

In this study, we hypothesized that the genetics-based interactions of the foundation tree species and one of its primary herbivores would influence the composition, abundance, richness, stability, and interaction network of a large multi-trophic community of associated arthropods. Unlike many previous studies of foundation species and other strong interactors, the importance of this study is that it experimentally examines how two strongly interacting species affect a large multi-trophic arthropod community. While there have been other studies showing that aphids have important community and ecosystem consequences (refs), and that cottonwoods can define community structure (refs), this study experimentally shows that the combined genetics-based interactions of the two have an even more pronounced effect on defining community structure (see related studies of Dickson and Whitham 1996; Schweitzer et al. 2005; Mooney and Agrawal 2008). If the genetics-based interactions of relatively few species define community structure is found to be generalizable to many systems, it would provide an important window on complex communities and ecosystems processes that otherwise might seem intractable. By focusing on the much smaller playing field of strongly interacting species rather than the whole community, we may account for a significant portion of the variation in community structure and stability.

Because tree genotype and environment were strongly controlled (aphid removal experiments and controls were done on the same tree), the fact that the presence of the aphid predictably altered all of these community traits is especially robust and is strong support for the IFS hypothesis. Most importantly, the fact that these community effects were tree genotype dependent based on the tree’s innate resistance to aphids, is strong support for a genetics-based IFS hypothesis. These findings demonstrate that complex community traits can be better understood using an interacting foundation species hypothesis and a community genetics approach. The results confirm that 1) The presence and abundance of a foundation herbivore has an effect on the resulting diversity and stability of the associated community, and 2) Genetic based variation in resistance to the herbivore indirectly influences the diversity and stability of large arthropod communities across years, and 3) That community composition and co-occurring network structure are determined by the interactions of two important species.

**Genetic basis of community stability**

While a genetic basis to community stability has previously been shown (Keith et al. 2010) our study further demonstrates the effect genetic based traits of foundation species can have on associated communities. If genetic differences in foundation species lead to more or less stable communities, then community properties such as diversity and stability can be put into an evolutionary framework and subject to natural selection. For example if important tree genotypes are lost, or founder genotype effects are introduced in restoration plantings, or the synchronous timing of bud break and aphid stem mother emergence are altered due to a selection event such as climate change, instability may be introduced into the system and result in significant effects on community traits and dynamics. Because the stability of communities is important in efforts to minimize loss of biodiversity (refs), the identification and conservation of foundation species interactions that affect complex community traits such as stability are increasingly important.

**Networks and interactions**

This study not only shows that the interactions of only two species can have far reaching effects on complex community traits such as diversity, stability but that the network structure of large communities can be influenced by an interaction between just two species. In addition to the previous work demonstrating the genetic basis of *P. betae* susceptibility and it’s community level impacts (Keith et al. 2010; Smith et al. 2011), the finding that the presence of *P. betae* tends to alter interaction network structure through the suppression of module formation and that the contribution of tree genotype to network modularity depends on a tree’s response to *P. betae* removal strongly supports the hypothesis that the interaction between these two foundation species is a driver of the structure of interactions in this community.

**Genetically based priority effects**

While population dynamics of *P. betae* vary from year to year along the Weber River, they are reliably present and repeatedly colonize the same genotypes year after year (Zinkgraf and Whitham unpub.). This is largely due to the phenological synchrony between these species, where aphid stem mothers are colonizing leaves as the leaves are flushing in order to lay eggs in the advantageous locations. Such consistent interactions provide a solid foundation upon which other species may find the resources they need. While priority effects in community assembly is a growing area of research in ecology, such a genetic basis to priority effects is unique and demonstrates that if the phenological synchrony between these species were to change a large dependent community may also fundamentally change.

**Conservation and Global Change implications**

In combination, these findings demonstrate that within a foundation species, variation in resistance to an associated foundation species can have far reaching community wide effects that determine not only the associated community’s structure but also the resulting diversity and stability of that community. If genetic variation within a species does, through interacting with other important species, determine the diversity and stability of the associated community then these complex traits can now be accounted for in restoration and preservation efforts. By identifying tree genotypes that promote the diversity and stability of arthropod communities, land managers could potentially conserve important species networks and provide greater resiliency to shifting populations due to changing climate and/or disturbance.

Overall, our study shows that to gain a better understanding of complex community traits like diversity, stability and network structure, which may seem too complex to determine due to the high number of species present, an examination of the interaction of just two very important species may be all that is necessary. We also demonstrate that the network structure of communities may be under genetic control and therefore subject to natural selection. We know of no other studies that have examined how the genetically determined interaction of two or more foundation species affects the network structure of a large, multi-trophic arthropod community.

**Acknowledgements:**

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Mooney and Agrawal 2008

Bailey and Whitham 2007

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**Figures:**

**Figure 1**



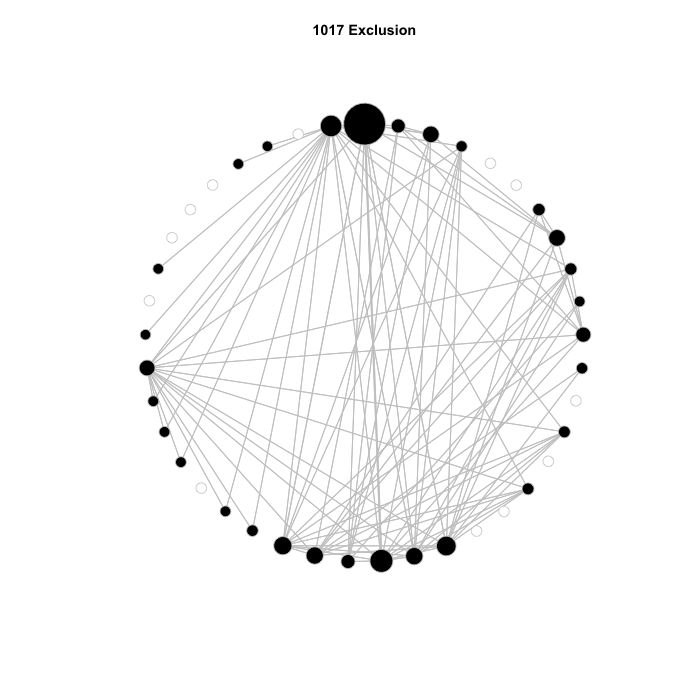
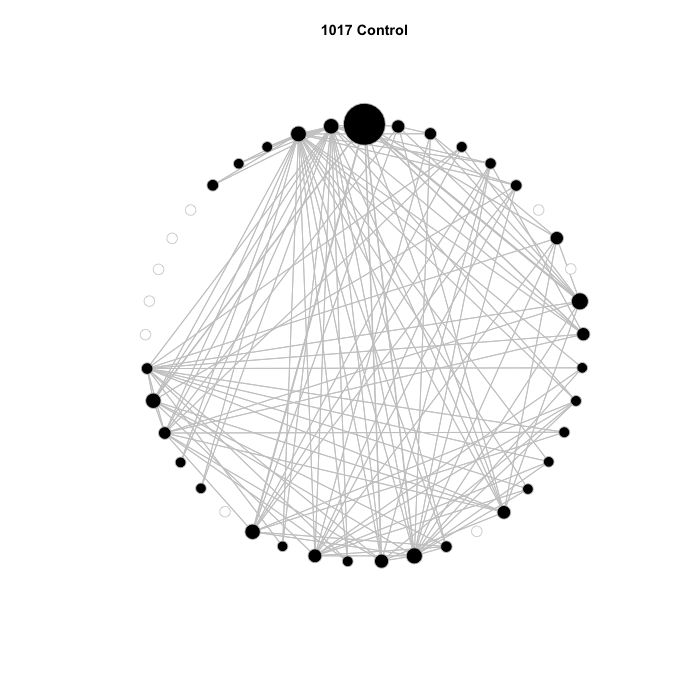
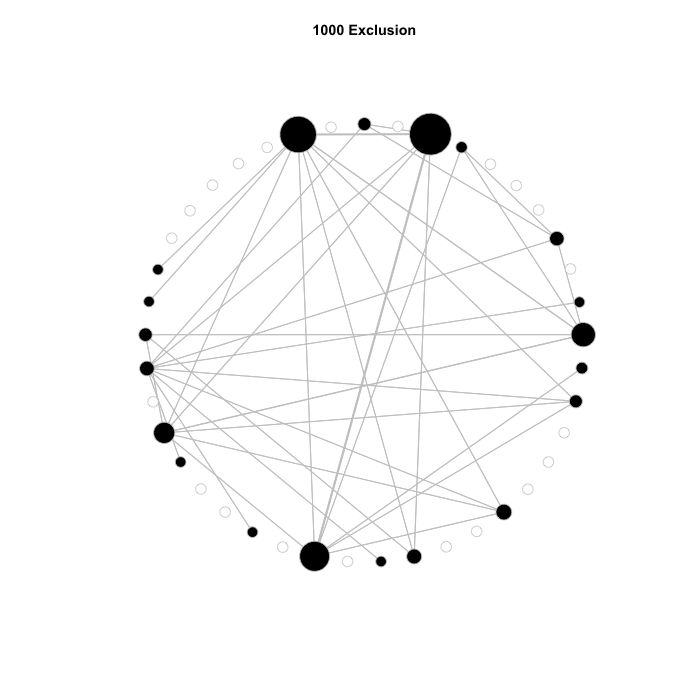
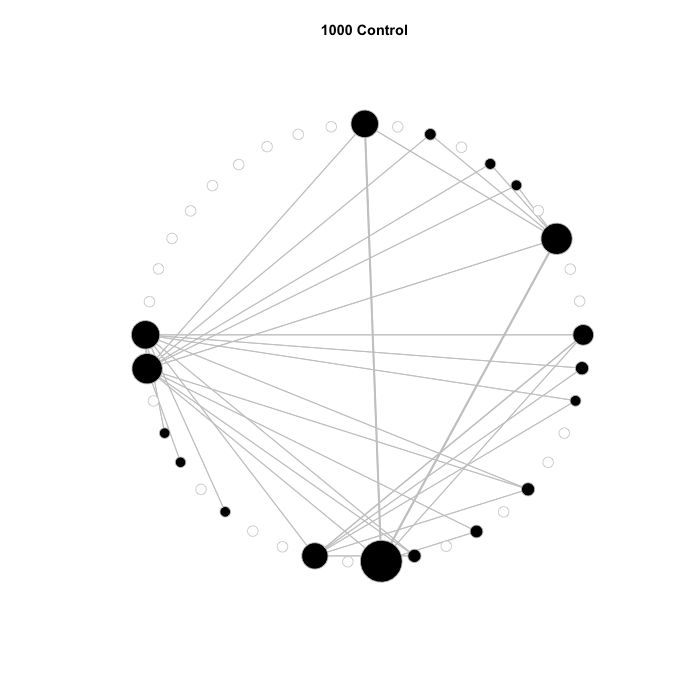
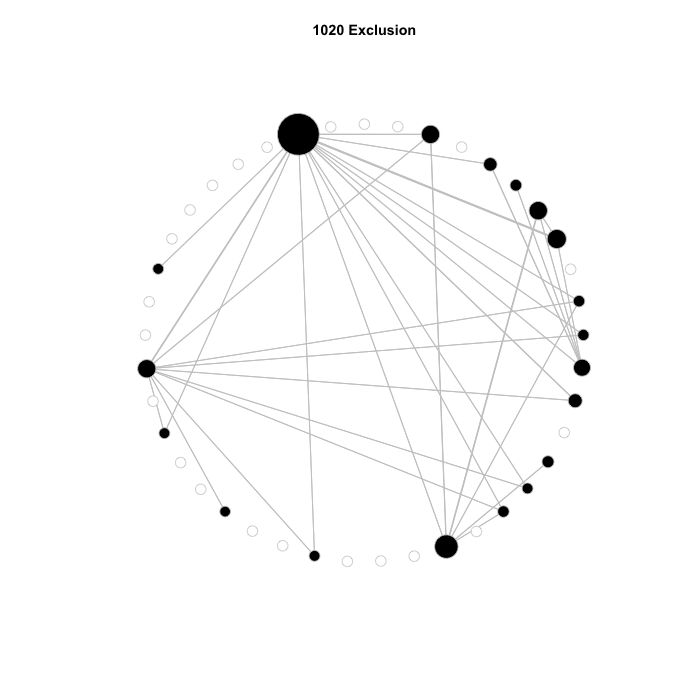
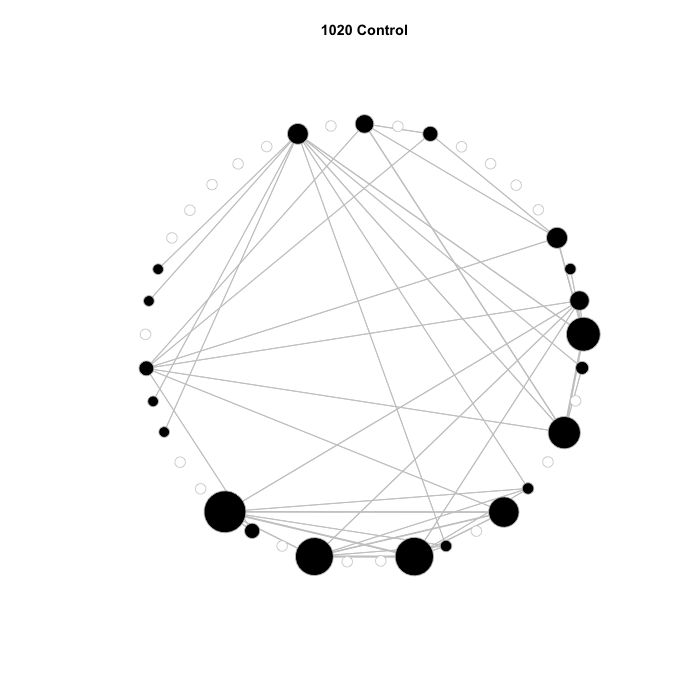
**Figure 2**



**Figure 3**



**Figure 4**



**Susceptible**

**Resistant**

**Aphid present**

**Aphid absent**

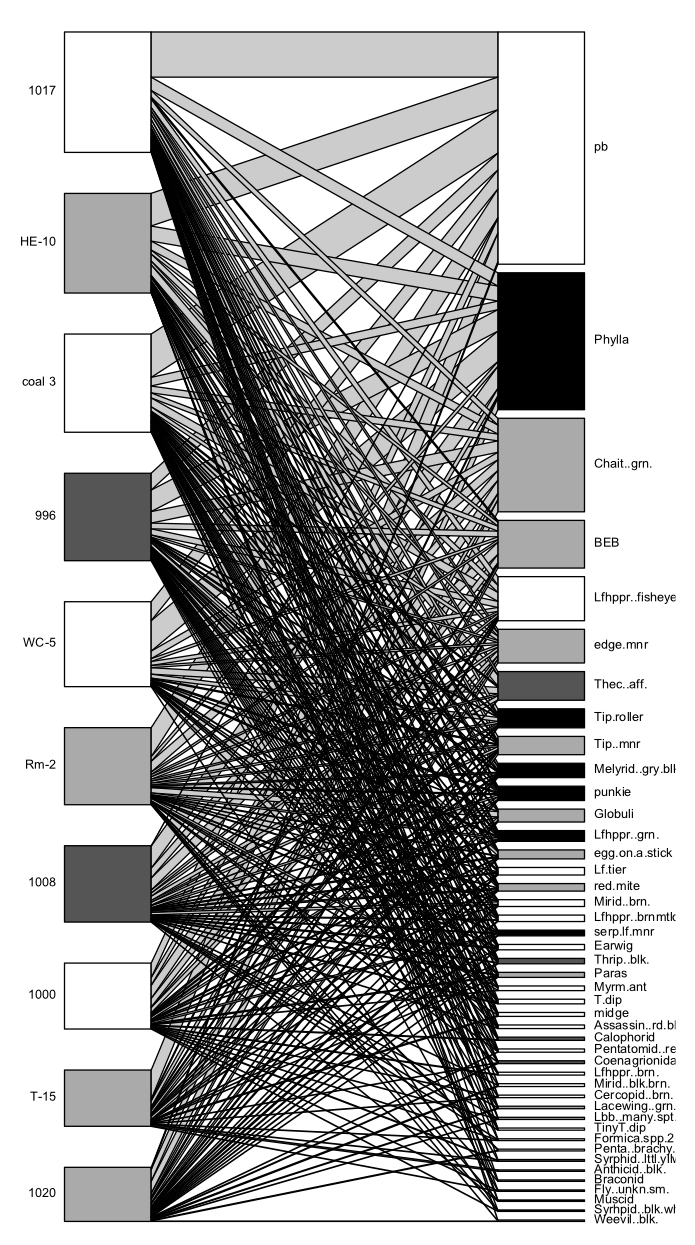
**More diversity**

**Less diversity**

**More stable**

**Less stable**

Or:



**Table of Arthropods:**