

# Evaluating the Effects of Aspen Genetics on Insect Communities

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## Abstract

Community genetics studies of plant-insect interactions have traditionally focused on bottom-up genotype effects of plants on insect communities. ...

## Introduction

Plant-insect interactions have traditionally been viewed through the lens of single species interactions (one plant and one insect) or interactions between whole communities. In particular, the effects of interspecific variation have been a focal point of community ecology in general (Power et al 1996) and in studies of plant-insect interactions. However, the contribution of intraspecific variation was largely ignored. In recent decades, more attention has been given to the effects that variation in individual species can have on entire associated communities (Des Roches et al 2017; Koricheva and Hayes 2018) and a recent meta-analysis showed that intraspecific trait variation contributed significantly (25%, on average) to plant community trait variation world-wide (Siefert et al 2015).

The interest in single-species effects on communities led to the development of a new field of study: community genetics. Proposed by Antonovics (1992), community genetics explores the effects of intraspecific variation on communities. This approach provides a means to understanding plant-insect interactions more completely than historical approaches. Research in this field has determined that different plant genotypes can have a strong influence on the composition and diversity of associated insect communities by way of phenotypic trait expression (Whitham et al 2003, 2008; Wimp et al 2005; Bangert et al 2008; Meneses et al 2012; Gosney et al 2017). **However, specific phenotypic traits, and suites thereof, that most influence insect communities remain poorly understood.** Even relatively simple insect communities, with only a few constituent taxa, are likely impacted by many host plant traits. Yet most plant-insect community genetics studies focus on only genotype differences and broad-sense heritability of communities without investigating the mechanisms that drive those differences. Studies in which phenotypic trait data have been collected have considered only a few traits, leaving an incomplete understanding of the complete phenotypic mechanisms through which genetic differences act on communities (Hersch-Green et al 2011; Crutsinger 2016).

To address phenotypic mechanisms of genotype-based community effects, we aim to evaluate the combined effects that plant genetics and traits have in shaping associated insect herbivore communities, using a *Populus* experimental system. In one step towards this end, we conducted a model-based, genome-wide association analysis of *Populus tremuloides*. We evaluated the effects of genotype at various SNP genomic markers on incidence of 18 common insect species, accounting a variety of traits and environmental factors that are known to affect insect communities.

## Background

### Community genetics: state of the field

The success of herbivorous insects is largely determined by physical and chemical characteristics of their host plants, many of which are genetically mediated (heritable). Even before the advent of community genetics, ecologists recognized that species genetics can influence communities. These effects were termed extended phenotypes (EP) (Dawkins 1982; Antonovics 1992), a term that is still used by community genetics investigators (Whitham et al 2003). Though plant genes do affect insect communities, they do not do so directly as in typical phenotypic expression. Instead, communities respond to plant phenotypes that are mediated by genes. Phenotypic traits, then, are the mechanisms of community genetic effects (Hersch-Green et al 2011; Crutsinger 2016).

Additionally, expression of individual traits can be constrained by other traits. High levels of expression in one trait can predispose a plant to low expression of a different trait. Co-expression of chemical defense and growth, for example, are limited by allocational and genetic costs (Sampedro 2014; Eichenberg et al 2015; Züst and Agrawal 2017). Therefore, the interaction of heritable plant traits, in addition to individual traits, may be key to shaping insect communities.

Many studies have shown genotype-mediated differences among plant-associated insect communities (Whitham et al 2003; Johnson and Agrawal 2005; Bangert et al 2006; Wimp et al 2010) but few have incorporated genetics and phenotypic expression to do so. Fewer still have used a wide range of naturally co-occurring genotypes and respective phenotypic variation. Studies meeting these criteria are needed to understand the capacity of a natural system for community genetic effects (Crutsinger 2016). Additionally, specific plant phenotypes and genotypes to which insect communities are most sensitive have not been investigated thoroughly in a community genetics context (with notable exceptions for phenotypes such as chemical defenses: see Gosney et al, 2017) (Hersch-Green et al 2011; Crutsinger 2016). Chemical, physical, and phenological plant traits, the genetic information that regulates these traits, and their interactions, all influence the structure and composition of associated insect communities. It is, therefore, important to investigate the effects of multiple plant traits with high levels of intraspecific variation. Furthermore, it is important to study plants from a population of genetically variable and naturally co-occurring genets of a foundation species such as aspen.

## **Populus tremuloides as a study system**

Trembling aspen is an ideal species for studying the interactions between genetics, traits, and insects because of its role in shaping insect communities. Community genetic effects of plants in a system are most likely to occur when: 1) The plant is a foundation species in the system, 2) the plant species has high levels of heritable variation in many traits, and 3) communities (insects) that are associated with the plant have many potential members that are differentially sensitive to plant traits (Antonovics 1992; Bailey et al 2006; Whitham et al 2006; Bangert et al 2008; Crutsinger 2016). Aspen satisfy all these criteria.

Aspen are considered a foundation species in North America and support some of the most biologically diverse communities in the US (Mitton and Grant 1996; Kay 1997; Madritch et al 2009; Kuhn et al 2011). The importance of aspen as a driver of biodiversity is even more pronounced when the scale at which it acts is considered; aspen is the most widely distributed tree species in all of North America (Little and Viereck 1971; Mitton and Grant 1996). Their extensive range necessitates interactions with a variety of different communities and locales and their high biomass potential allows them to support large communities.

Aspen also exhibits a tremendous amount of phenotypic trait variation (Mitton and Grant 1996; Donaldson et al 2006; Lindroth and St Clair 2013) which provides an opportunity to study consequences of intraspecific variation for insect herbivore communities. Variation in traits means variation in quality and quantity of aspen as a food resource, which leads to variation in performance and abundance of aspen-associated insect herbivores (Hwang and Lindroth 1997; Lindroth et al 1999, 2007; Meneses et al 2012).

Chemical defenses, for example, are among the most well-studied heritable aspen traits, in terms of variation and significance, that affect insect performance and influence communities (Erwin et al 1994; Lindroth et al 1999, 2007; Wimp et al 2007). Two predominant classes of aspen secondary metabolites are condensed tannins (CTs) and phenolic glycosides (PGs). Some variation in aspen traits such as PG and CT concentrations may be attributed to allocational, genetic, or phenological costs to other traits and phenotypic plasticity (Stevens and Lindroth 2005; Osier and Lindroth 2006). An example of plasticity can be seen with secondary metabolites of aspen which can be both constitutive and inducible (Osier and Lindroth 2001; Rubert-Nason et al 2015). This means that genotype and the environment (insect herbivores) interact to influence the expression of resistance compounds within a plant. Allocational costs to trait expression are likely contributors to the strong growth-defense tradeoffs documented in aspen (Hwang and Lindroth 1997; Donaldson et al 2005; Osier and Lindroth 2006; Cole et al 2016).

# Methods

## Experimental Design

**WisAsp population:** the Lindroth research group has established the “Wisconsin Aspen Genetic Mapping Population” (WisAsp), a common garden plantation of *Populus tremuloides*, in 2010. WisAsp provides a unique opportunity to use many genetically and phenotypically diverse genets of aspen to test potential natural insect community differences. WisAsp exhibits high variation in many traits, including tree size, phenology, and chemical composition. Additionally, a wide range of generalist and specialist insect associates have become established at the common garden, including taxa from at least 7 orders and 125 species (Morrow, unpublished data; Barker, 2015). Common feeding guilds of the insects present at WisAsp include leaf-chewing, leaf-mining, leaf-galling, phloem-feeding, and wood-boring insects. The diversity of insects that utilize the WisAsp trees provides an opportunity to test the effects of specific traits on specific insect guilds and taxa as well as to test the overall effects on complex communities.

The garden contains 517 genotypes, with an average of three clonal replicate trees (ramets) per genotype for a total of 1,568 experimental ramets. The experimental trees are buffered from the external environment by a border of 255 non-experimental trees. The trees from each genotype were planted as cuttings collected from a contiguous section of root material. Genotypes were collected from 13 counties throughout the state of WI. The experimental ramets were arranged in a randomized complete block design with 4 blocks. Replants were conducted in 2011 and 2012 to replace trees that died in previous years. These replants resulted in replacement of 147 trees belonging to different genets and a nonuniform age structure of the plot.

## Data collection

Data were collected on tree genetics, traits, and associated insect communities. Insect community data were collected by conducting visual surveys of insect herbivores on the lower third of each tree’s canopy. The survey boundary was chosen due to constraints imposed by the number of trees and large size of each tree as well as the short duration of the surveys. Species rarefaction estimates (Sanders 1968; Simberloff 1972; Gotelli and Colwell 2011) collected one week prior to the first insect survey showed no difference in species richness among the top, middle, or bottom thirds of the trees, justifying this census approach. Trees were surveyed for a predetermined duration, with 3-minute minimum and 10-minute maximum caps, based on relative height, to estimate insect density (as a function of time).

Of 104 total species of insects surveyed, 18 were identified as common (occurred on  $\geq 5\%$  of trees in each survey event) and will be used in the analysis. Phytochemical analyses were conducted on leaf tissue from each ramet at each insect survey event. PGs were extracted from leaf tissue and quantified using UHPLC-mass spectrometry following methods of Abreu, modified by the Lindroth Lab (Abreu et al 2011; F Rubert-Nason et al 2017). CTs were extracted and quantified following the methods of Porter/Hagerman (Porter et al 1985; Hagerman and Butler 1989). Carbon and Nitrogen concentrations were quantified using near infrared spectroscopy (NIRS) with nitrogen analyzer calibration (Rubert-Nason et al 2013). All phytochemical extractions were done on leaves that had been vacuum-dried for 48 hours. Other tree traits quantified (volume, basal area, number of flowers, sex, leaf area, bud break timing, and extra-floral nectaries) were done so using standard methods.

Coding-region genomic data (DNA) were collected for all Genets, (437 genets and 1569 trees remained after filtering). The sequenced reads were aligned to scaffolds of a known reference genome of *Populus tremuloides*. Absolute locations of these scaffolds in the genome are currently unknown (though work to resolve this is currently ongoing) and, therefore, only relative within-scaffold locations of each marker are certain. Among our population, 11420 SNP markers were identified, after applying a .05 minor allele frequency filter to remove potential false positives.

## Statistical Analyses

We conducted a model-based case/control genome-wide association (GWA) study of *Populus tremuloides* (aspen) and incidence of 18 common insect herbivore species. Incidence was measured on 1414 trees twice per summer (June and August) in 2016 and 2017 for a total of 4 surveys events. Among these trees, there are 437 unique individuals (genets), each with an average of 3 clonal replicates.

We used generalized linear mixed models (GLMM) to test the effects of aspen genotype (no. of alleles equal to reference allele), at 114420 SNP markers, on insect presence. Unlike traditional GWA methods, GLMMs allow for the ability to robustly test for genotype associations while accounting for aspen traits already known to influence insect communities, variation of those traits within a genet, and temporal variation of traits and insect communities. In total, 2059560 GLMMs were created, one for each insect by SNP combination. This required. These models were built and executed using `lme4`, a mixed model package for the R statistical software, and high-throughput computing resources (distributed computing) at the University of Madison - Wisconsin.

The DNA reads of each tree were aligned to scaffold regions of the reference genome. Genomic position relationships among and within chromosomes is currently unknown for aspen. Therefore, locations of SNPs are only understood as relative location within scaffolds. For this reason, SNPs were not pruned upstream for LD. Even with this limitation, Genes and expression annotations for *Populus trichocarpa* and *Arabidopsis* can be imputed to a list of aspen SNPs to draw conclusions about function.

## GLMM

We consider a generalized linear mixed model (GLMM) for GWA. For each SNP, denote  $p_{ijk_g}$  as the probability of observing non-zero count of insect type  $i$  on tree  $k$ , belonging to genotype  $g$ , during survey event  $j$ , the GLMM for a SNP has form:

$$\text{logit}(p_{ijk_g}) = \beta_0 + \alpha G_g + x_{jk_g}^T \beta + \varepsilon_{g(j)}$$

where  $\text{logit}(y) = \log\left(\frac{y}{1-y}\right)$ ,  $G_g$  is the SNP-specific genotype of genet  $g$ . Genotype is defined here as the number of alleles that match the reference allele at the SNP location, i.e. additive coding of genotype is adopted. Moreover,  $x_{jk_g}$  is the vector of observed tree trait covariates, which includes volume of the tree, average leaf area of the tree, standardized leaf area of the tree, degree days at which the tree's leaves became fully opened, average extra-floral nectaries per leaf of the tree, foliar condensed tannin concentration, salicinoid phenolic glycosides concentration, and age of tree. Since clonal replicates are nested under the four survey events, we consider  $\varepsilon_{g(j)}$  as a random effect to introduce dependence between observations with same clonal replicate at the same survey period. To conduct GWA, the two-tailed t-test p value of  $\alpha$  in the GLMM is used to identify significant associations after fdr correction.

## Computation

The R statistical software is used for computing. Due to the nested random effect of the model, we use `lme4::glmer()` to fit the GLMM. Moreover, the computing task is parallelized using the Center for High Throughput Computing (CHTC) under the Department of Computer Sciences at the University of Madison - Wisconsin.

We then converted our p values into q values (Storey), controlling for a false discovery rate of .05. Then, for discovery purposes, we use a .05 significance level cutoff for q values to identify significant associations.

# Results

## Significant Associations

From the 114420 total SNPs, we identified 4768 unique SNPs with significant associations ( $q < .05$ ) to incidence of at least one of our common insect species. Those SNPs were located on a total of 2042 scaffolds. Table 1 shows the breakdown of significant associations by all of our common insect species.

Table 1: Insect descriptions, number of significant SNP associations per insect, and number of scaffolds on which those SNPs are located.

	insect description	SNPs	scaffolds
Green Aphids	free-feeding, specialist (salicaceae)	1788	1020

	insect description	SNPs	scaffolds
Petiole Gall	leaf-galling, specialist (populus)	1432	788
Phyllocolpa	leaf-rolling, specialist (salicaceae)	1363	814
Harmandia	leaf-galling, specialist (populus)	174	130
Smokey Aphids	free-feeding, specialist (populus)	90	64
Casebearer Moth	case-bearing, generalist	7	3
Lombardy Mine	leaf-mining, specialist (populus)	1	1
Cottonwood Leaf Mine	leaf-mining, specialist (salicaceae)	1	1
Leaf Edge Mine	leaf-mining, specialist (salicaceae)	0	0
Blotch Mine	leaf-mining, specialist (populus)	0	0
Weevil Mine	leaf-mining, specialist (salicaceae)	0	0
Blackmine Beetle	leaf-mining, specialist (populus)	0	0
Leafhoppers	free-feeding, generalist	0	0
Ants	aphid-tending, non-herbivore	0	0
Pale Green Notodontid	free-feeding, specialist (populus)	0	0
Aspen Leaf Beetle	free-feeding, specialist (populus)	0	0
Green Sawfly	free-feeding, specialist (populus)	0	0
Cotton Scale	scale insect, generalist	0	0

Of the 18 common insects, 8 had significant associations. Among those, 84 SNPs were significantly associated with at least 2 insects (figure 1). Because we are interested the effects of the aspen genome on insect communities, these 84 SNPs with shared significance will our SNPs of interest. These insects are expected to affected by similar mechanisms, due to their similar feeding strategies (table 1). Therefore, it is more likely that those SNPs that affect multiple insects are truly biologically significant. There is especially strong evidence for 4 SNPs which were associated with 3 insect species and are located in the same genomic region (table 2). This indicates that a true loci of interest lies within this genomic region.

For Supplemental Materials see: sup. materials.

Table 2: q values for SNPs with 3 associations. All SNPs are located on the same scaffold and are within 63 bases. They are almost certainly in LD but suggest that there may be an important locus in the region.

SNP	Harmandia	Phyllocolpa	Petiole Gall
Potra002191:26587	0.0193784	0.0123563	0.0286529
Potra002191:26642	0.0226734	0.0091460	0.0258001
Potra002191:26644	0.0226734	0.0091460	0.0258001
Potra002191:26650	0.0236877	0.0116280	0.0234547

## Discussion

### Future Directions

Due to resource and time limitations, we were unable to perform thorough model selection steps with SNPs included, however the model that was selected had among the best performance of similar models without SNP terms. Further model selection steps should be done to determine if a better model exists. We were also unable to compare this method to traditional approaches. We would like to re-run the analyses in **plink** (without random effects) and compare results. We believe that this method provides a more robust method of identifying significant associations than simple linear regression techniques. In fact, we were able to identify far more significant associations with this method than a similar study on the same common garden (and the same insects) in 2015 that used the traditional approach (Barker et al 2018).

We also have not yet completed gene imputation for our list of interesting SNPs. This step is crucial to

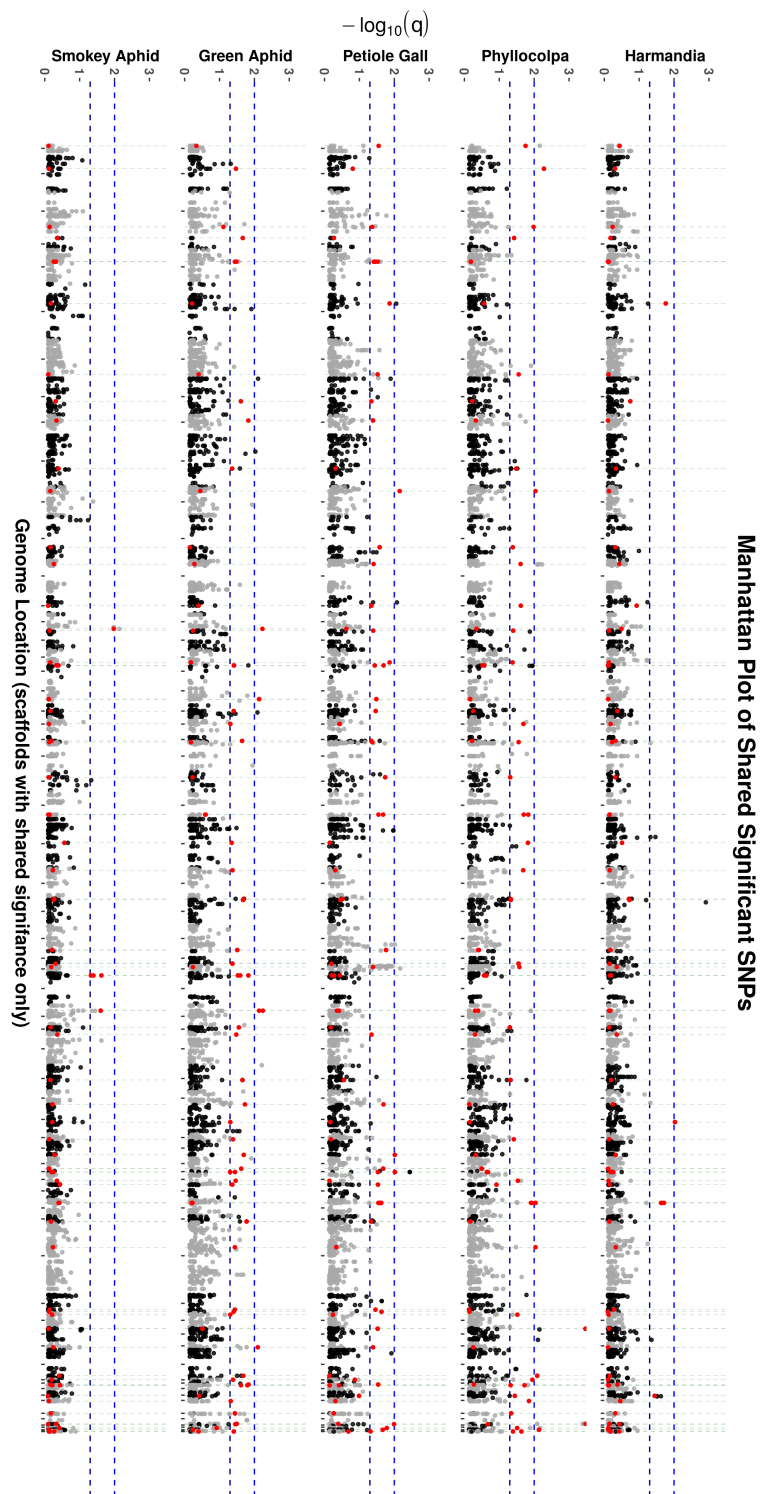


Figure 1: Manhattan-style plots of 5 insects that share significant SNPs. The x-axis shows a filtered genomic region; only scaffolds with shared-significant SNPs (red) are shown.  $q$  value based LOD scores are shown on the y-axis with dashed lines representing significance levels of .05 (dark blue) and .01 (blue). When an insect has a red SNP above the .05 line, it shares this SNP association with another insect. Note that adjacent scaffolds on the x-axis (alternating black and grey) do not correspond to adjacent regions in the genome. Exact locations of scaffolds in the genome are currently unknown.

understanding the phenotypic mechanisms that are driving differences in insect incidence due to genotype. This will also help us to further determine if our significant associations make biological sense. This is the next step for this project.

Finally, these GWA results fit into a larger project that aims to understand the intraspecific variation of aspen phenotype (including insect communities and tree traits). With the larger study we are attempting to explain variation in insect communities among aspen genets using measured traits and environmental factors. The genotype associations will give us a better understanding of the genet-driven variation of insect communities that remains unexplained by these measured traits.

## References