# Linking plant genetics and environment to associated insect species and community composition

By

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## Thesis introduction

In the past decade, ecological research has highlighted the importance of intraspecific variation in influencing species interactions, community dynamics, and ecosystem processes (Whitham et al. 2008, Schweitzer et al. 2008, Zytynska and Preziosi 2011, Koricheva and Hayes 2018). Intraspecific variation can be as important and in some contexts, even more important than interspecific variation in ecological systems (Des Roches et al. 2018). In particular, intraspecific variation in foundation plant species can structure communities of associated arthropods, pathogens, lichens, endophytes, understory plants, and predators (Bangert et al. 2006, Wimp et al. 2007, Lamit et al. 2011a, 2011b, 2014, Busby et al. 2013) and shape ecosystem processes, including nutrient cycling (Madritch et al. 2009).

Intraspecific variation at the phenotype-level is shaped by three factors: genetics, environmental context, and the interaction between genetics and the environment (GxE). The heritable component of a phenotype reflects the capacity for trait evolution, while plasticity (environmental and GxE components) measures the capacity of an organism to respond to and/or tolerate environmental stressors (Falconer and Mackay 1996, Hendry 2016). In foundation plant populations, ecologically-relevant traits vary in both heritability and plasticity (Geber and Griffen 2003, Lindroth et al. 2007, Wooley et al. 2007), yet these findings are rarely quantified and summarized across studies to identify general patterns.

Community genetics aims to understand the effects of intraspecific genetic variation on community composition and diversity, thereby connecting community ecology with evolutionary biology. To this end, the study of plant systems has

investigated how different genotypes of plant species have analogously different communities of associated organisms (*e.g.,* arthropods; Busby et al. 2014, Barbour et al. 2015, Koricheva and Hayes 2018, Kagiya et al. 2018). Furthermore, the associated communities are heritable, which implies that related plants have more similar associated communities than distantly related plants (Bangert et al. 2006). Indeed, associated communities can be thought of as an extension of the plant's phenotype ('extended phenotype', Whitham et al. 2003). This heritability also indicates that particular plant genes and traits are important in shaping these dependent communities, yet both the identity of these genes and the relative importance of plant traits in structuring communities remain largely unknown (Crutsinger 2015).

This research addresses several key gaps in our knowledge of the effects of intraspecific variation in plants in shaping plant traits and associated insect communities:

1. In a quantitative literature review, I partitioned Salicaceae (*Populus* and *Salix*) phenotypic variation into genetic, environmental, and genotype by environment interactions to determine how these sources of variation compare across plant phenotypes, extended phenotypes (*i.e.,* insect performance), and environmental conditions.
2. I determined the relative importance of different trembling aspen (*Populus tremuloides*) traits (*i.e.,* morphology, phenology and phytochemistry) in shaping canopy insect communities.
3. I identified *Populus* genes and gene functions/processes that underlie plant traits and canopy insect populations and community structure.

Salicaceae (*Populus* and *Salix*) provides an ideal system for this work due to its wealth of genetic resources and ecological importance. *Populus* is currently *the* model tree species for genetics (Jansson and Douglas 2007, Cheng and Tuskan 2009). Accordingly, there are many large *Populus* breeding populations grown in multiple environments and available genetic maps and online resources including the *P. trichocarpa* annotated genome (Tuskan et al. 2006, Cheng and Tuskan 2009). In addition, Salicaceae taxa have excellent population characteristics for genetic association analyses to connect particular genes to ecological phenotypes (*e.g.,* low population struture; Ingvarsson 2010). Salicaceae species are also widely distributed in temperate regions and several are foundation species that structure associated communities (Robinson et al. 2012) and surrounding ecosystems (Schweitzer et al. 2008). Both *Populus* and *Salix* have been studied in genotype by environment interaction experiments (Lindroth et al. 2002, Weih et al. 2006, LeRoy et al. 2012) and community genetics research (Wimp et al. 2007, Robinson et al. 2012, Barbour et al. 2015).

My dissertation research consists of a meta-analysis of the Salicaceae literature and a genetic mapping study using trembling aspen (*Populus tremuloides*). Aspen genets (N = 445) for the genetic mapping study were collected in 2010 from throughout Wisconsin, replicated from rootstock, and planted in a common garden in the Arlington Agricultural Research Station near DeForest, WI. This garden is called “WisAsp” for Wisconsin Aspen, and the overarching goal of this project is to identify the underlying genes driving variation in ecologically-relevant tree traits and canopy insect communities.

My first chapter reviews the Salicaceae literature to quantify and partition phenotypic variation (*i.e.*, plant growth/size, phytochemistry, and insect herbivore performance [an extended phenotype]) into genetic and environmental components and their interaction (GxE). Using meta-analysis methods, I surveyed the literature finding 74 articles that were suitable for inclusion in my analysis. These studies used controlled manipulations or replicated common gardens to determine the effects of an environmental treatment (*e.g.,* amended soil nutrients, enhanced ozone) and plant genetic variation (population of different genets or hybrids) in shaping plant trait variation and variation in insect herbivore performance. I then used multilevel mixed models to summarize variation estimates (G, E, and GxE) across studies and factors of interest (*e.g.,* trait type, environmental condition, insect herbivore group).

My second chapter quantifies aspen trait variation and canopy insect communities to reveal how the insect communities were structured (*e.g.,* relationships among insect groups, community heritability) and shaped by tree traits. I collected tree trait information (*i.e.,* tree size, leaf morphology, phytochemistry, bud phenology, extra-floral nectaries) and visually surveyed canopy insects in both 2014 and 2015 at WisAsp. I then used mixed models to derive community heritability estimates across insect community metrics (*e.g.*, richness, total abundance) and groups (*e.g.,* families, functional groups) and to determine which tree traits influenced insect community composition.

My third chapter identifies the aspen genes and gene processes/functions that were associated with ecologically-relevant tree traits and canopy insect communities. I used the tree phenotype and insect data from my second dissertation chapter along with genetic data from each WisAsp genet for genome-wide association analyses. Each aspen genet was sequenced with 45,934 probes that aligned to gene regions; from this information, 173,520 single-nucleotide polymorphisms (SNPs) were identified for analyses. I used both genome-wide association models and gene set enrichment to identify genes and gene functions that strongly correlated with aspen traits and canopy insects.

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# Chapter One

## Independent and interactive effects of plant genotype and environment on plant traits and insect herbivore performance: a meta-analysis with Salicaceae

Chapter One is currently in preparation for submission to one of a few potential journals, including Functional Ecology, Evolutionary Ecology, and Oecologia.

## Introduction

*Darwin was obsessed with variation. His books, considered as an ensemble, devote much more attention to variation than to natural selection, because he knew that no satisfactory theory of evolutionary change could be constructed until the causes of variation and the empirical rule of its form and amount had been elucidated (Gould 1983).*

Exploration of the causes and consequences of biological variation at the interspecific level has long been a central focus of evolutionary ecology. Over the last decade, research has increasingly highlighted the importance of variation at the intraspecific level in shaping communities and ecosystems (Bolnick et al. 2011, Violle et al. 2012, Koricheva and Hayes 2018). Intraspecific variation has been shown to be comparable to, and in some cases even more important than, interspecific variation (Des Roches et al. 2018). In plant populations, intraspecific variation can have far-reaching effects, from structuring associated arthropod, endophyte, and secondary consumer communities (Lamit et al. 2014, 2015, Koricheva and Hayes 2018) to governing ecosystem processes, such as nutrient cycling and primary production (Crutsinger et al. 2006, Hughes et al. 2008, Madritch et al. 2009).

Previous work has emphasized the importance of intraspecific variation at the phenotypic level (Bolnick et al. 2011, Siefert et al. 2015), which is comprised of genetic (G) and environmental (E) components, and their interaction (GxE, but see Geber and Griffen 2003). While selection operates on phenotypes, the genetic components of a trait (G and GxE) influence evolutionary trajectories. Traits with larger genetic variation can respond more readily to selection (Falconer and Mackay 1996), while plasticity can both advance and restrict evolution depending on the context (Hendry 2016). Here, I partition plant phenotypic variation into these components to ascertain differences in the

partitioning of variation across plant traits, environmental contexts, and interactions with insect herbivores.

A critical challenge confronting plant populations is the capacity to respond to a spatially and temporally variable environment. Recent work indicates that plant populations have responded to global change via both phenotypic plasticity and evolutionary processes, yet these responses may not be sufficient to keep pace with changing climate (Franks et al. 2014, Hendry 2016). In addition, changing biotic environments, especially shifts in the distribution and intensity of insect pests, represent a serious threat to plant populations (*e.g.,* Raffa et al. 2008). The capacity of plants to evolve resistance or tolerance to these herbivores is determined by the extent to which plant genetic variation shapes insect herbivore performance (*e.g.,* Carmona et al. 2011).

The purpose of this meta-analysis was first to quantify the contributions of plant genetic, environmental, and GxE components to phenotypic variation, and second, to assess *variance* rather than *means*. An important limitation of previous meta-analyses (Koricheva et al. 1998, Valkama et al. 2007, Dios et al. 2016, Li et al. 2016, etc.) is their focus on changes in the *mean effect, rather than variance,* of plant traits and insect responses in changing environments. As described previously, assessment of such variation is critically important for understanding plant biology at the levels of populations, communities and ecosystems, and over both ecological and evolutionary time scales. To date, no review has determined the extent to which plant genetics, environment, and their interaction shape plant phenotypic *variation* and associated variation in insect performance. To address this void, I reviewed the existing literature on the effects of plant genotype, environmental context, and genotype x environment

interactions in shaping plant phenotypic variation and insect herbivore performance. I compared and contrasted a comprehensive set of environmental factors (*e.g.,* site characteristics, soil fertility, water, temperature, light, competition), plant traits (*i.e.,* plant growth, foliar defense compounds, foliar nitrogen levels), and insect herbivore performance metrics (*e.g.,* fecundity, survival, development, growth).

I focused on Salicaceae because of the large body of published research available on genotype by environment interactions in *Salix* and *Populus*, and because of the taxa's significant ecological, genetic, and economic value. Salicaceae is broadly distributed in temperate regions, and many taxa within the family are foundation tree species (*e.g.,* cottonwoods and aspen) that play important roles in structuring their surrounding ecosystem and dependent communities (*e.g.*, arthropods, Keith et al. 2010, Bernhardsson et al. 2013). In addition, *Populus* species have become the premier genetic model for forest trees, with an array of available genetic resources (*e.g.*, annotated genomes; Cronk 2005, Sjödin et al. 2009). Salicaceae species are frequently used in bioenergy production, soil remediation practices, and paper pulp production (Tognetti et al. 2013, Banday et al. 2017). They also have a unique set of well-studied defense compounds, salicinoids, which are derived from the shikimate-phenylpropanoid pathway (Boeckler et al. 2011).

In this quantitative review, I address the following questions: (1) What are the relative contributions of genotype, environment, and genotype x environment interactions in Salicaceae that shape the variation of particular traits (growth and phytochemistry) and associations with insects? (2) What are the relative strengths of different environmental variables in shaping phenotypic variation? (3) How does

phenotypic plasticity vary across plant traits and environmental contexts? (4) To what extent do plant genetics alter the variation in insect herbivore performance? Does this contribution vary across insect orders and across specialist versus generalist insect feeders?

## Methods

### *Literature search*

To identify relevant records for my database, I conducted multiple searches with three search engines, Google Scholar, Index to Theses Online, and JSTOR. I used a combination of search terms, including “Salicaceae”, “*Populus*”, or “*Salix*” and “genotype environment interaction” or “genetic environment interaction” for a total of 18 searches resulting in 1254 records after deleting duplicates (Fig. 1). The final search was performed on August 21, 2017.

These records were then screened for my inclusion criteria. For inclusion in my meta-analyses, these records had to study (1) a plant species in the Salicaceae (*Populus* or *Salix*) with treatments that assessed both (2) intraspecific genetic variation (*e.g.,* testing multiple replicated genotypes, breeding populations, hybrid crosses) and (3) an environmental variable (*e.g.,* testing the same population at different sites, soil nutrients, temperature, water, etc.). In addition, these studies had to (4) assess at least one of the following response variables: tree growth or size (*e.g.,* stem volume, biomass, relative growth), phytochemistry (*i.e.,* levels of condensed tannins, phenolic glycosides, other phenolics, nitrogen), or insect herbivore performance (*e.g.,* fecundity, population growth, survival, feeding). Finally, the record had to be a primary study with

an appropriate design (*e.g.,* replicated breeding populations, replicated genotypes in different environmental conditions) and (5) include either ANOVA statistics (*e.g.,* F statistics, degrees of freedom) or variance components that partitioned the effects of genotype/clone (G), environment (E), and their interaction (GxE) on the variation in the response variable. Finally, records (N = 6) that were not in English were excluded.

After filtering, 72 studies published during 1968-2017 with 379 sets of G, E, and GxE statistics met all inclusion criteria (Supplemental Table 1), while an additional 50 studies met all criteria except that they did not include the appropriate statistics (Fig. 1). These 50 excluded studies had a similar distribution of study system (*Populus* or *Salix*), response variables (except there were more experiments that measured tree growth and fewer that measured insect herbivore response), and environmental treatments (except there were more experiments that manipulated ozone and water levels) compared with the included studies (Fig. 1). Of the 72 included studies, three were theses.

From the 72 included records, I extracted study-level data and information to calculate variation estimates. I recorded the study location (city, state/province, and country), study design (potted, field or greenhouse experiment), year of publication, and publication journal. In addition, I recorded the age of the trees when measurements were taken, number of replicates for each genotype x environment combination, the genus (*Populus* or *Salix*) and primary species studied (for studies that included hybrid crosses, I recorded the two most common species in the population), number of genotypes/hybrids, and the total number of species within the population. If the study included an insect herbivore response, I also recorded the insect's order, genus, and

species, functional group (free-feeder vs. leaf-modifier), diet breadth (specialist or generalist), and whether the insect was exotic or native.

My "effect size" was generalized eta square ($\eta^2$; Fritz et al. 2012, Trigo Sánchez and Martínez Cervantes 2016), a metric that quantifies proportions of variation and ranges between zero and one. In contrast to Hedge's D, a common metric in meta-analyses, $\eta^2$ is derived from ANOVA outputs (sum of squares) rather than from summary statistics (means and standard deviation, Koricheva et al. 2013). I calculated generalized eta squared ($\eta^2$) as:

$$\eta_G^2 = SS_G \,/(SS_G + SS_E + SS_{GxE} + SS_e)$$

$$\eta_E^2 = SS_E \,/(SS_G + SS_E + SS_{GxE} + SS_e)$$

$$\eta_{GxE}^2 = SS_{GxE} \,/(SS_G + SS_E + SS_{GxE} + SS_e)$$

where *SS* is the sum of squares for the various components that comprise phenotypic variation, including plant genotype ($SS_G$), environmental treatment ($SS_E$), genotype by environment interactions ($SS_{GxE}$) and the model error ($SS_e$). Thus, calculating $\eta^2$ is analogous to calculating the coefficient of determination ($R^2$) in regression analysis. The main difference is that the denominator variance includes only the factors of interest that are comparable across studies (*i.e.,* G, E, and GxE sum of squares, error), and does not include study-specific sources of variation (*e.g.,* block, species, co-variates). In addition, the generalized eta squared estimate for plant genetics ($\eta_G^2$) is comparable to broad-sense heritability. Generalized eta squared has been widely used in the psychology literature, and a similar variation metric has been used in an ecology meta-analysis (Tack et al. 2012).

*Meta-analysis*

I used multilevel mixed models with the metafor package in R (Viechtbauer 2010) for meta-analyses. Multilevel models account for non-independence among estimates from the same study. I weighted the logit-transformed variation estimates ($\eta^2_G$, $\eta^2_E$, $\eta^2_{GxE}$) by the number of replicates for each genotype x treatment combination (*i.e.,* study precision). To account for heterogeneity across studies, I used meta-regression with model selection (based on Akaike Information Criteria, AIC) to determine which study-level covariates (*e.g.,* age of trees, study location, publication year, Salicaceae genus, etc.) should be included in each model (see Table 1 for a summary of each model). The extent to which genetic, environmental and genotype by environment (GxE) components shape phenotypic variation is population- and context-dependent and can vary with the genetic makeup of the population and the environmental range that the individuals experience (Falconer and Mackay 1996). Thus, to account for these factors, I included both the number of species and genets (which indicates allelic variation) within the Salicaceae populations and the ratio of the number of species/genets to the number of levels of the environmental treatment (which indicates the extent of the allelic and environmental variation) as potential covariates for model selection.

To assess the extent to which variation estimates ($\eta^2_G$, $\eta^2_E$, $\eta^2_{GxE}$) differed across studies, I quantified residual heterogeneity with Cochran's Q-test. To determine how my modeling design influenced the results (*i.e.,* sensitivity analysis), I reanalyzed my models in two ways: without using a multilevel structure and without influential variation estimates (N = 14, these influential estimates were mainly $\eta^2_{GxE}$ and $\eta^2_E$ estimates that

were close to zero). I did not include phylogenetic controls since this was a targeted meta-analysis that focused on only Salicaceae species.

To assess potential bias in the data, I investigated temporal changes in variation estimates and publication bias. To evaluate temporal changes, I regressed generalized eta squared (separately for $\eta^2_G$, $\eta^2_E$, $\eta^2_{GxE}$) on the year of publication. To assess publication bias I used contour-enhanced funnel plots of the variation estimates and the models' residuals after detrending the $\eta^2$ values by study-level covariates (Nakagawa and Santos 2012). Publication bias is indicated if funnel asymmetry is present in the data.

**Results**

Heterogeneity among variation estimates ($\eta^2_G$, $\eta^2_E$, $\eta^2_{GxE}$) was considerable across studies, even when study-level attributes, such as environmental treatment and tree age, were controlled (Table 1, Supplemental Table 2). While the observed values of genetic, environmental and GxE variation for Salicaceae traits showed significant publication bias (*i.e.,* suggesting that studies with insignificant genotype, environment and/or GxE variation components may be lacking from the published literature), these values did not appear to be biased by publication trends after simple moderators (*e.g.,* environmental treatment, study ID) were taken into account (Fig. 2). In addition, (logit-transformed) $\eta^2$ values did not change with year of publication, indicating no bias over time (Fig. 3).

*Question 1: Relative effects of G, E and GxE across traits*

Genetics (G), environment (E) and their interaction (GxE) varied in the degree to which they contributed to phenotypic variation in Salicaceae traits and insect herbivore performance metrics (Fig. 4). Variation in both plant growth and foliar nitrogen was more environmentally- than genetically-determined. In contrast, defense compounds (salicinoids and condensed tannins) were influenced much more strongly by genetics than by environment. Variation in insect herbivore performance was shaped primarily by host plant genetics, although this $\eta^2_G$ estimate was variable across studies (*i.e.,* wide confidence interval). The amount of variation that derived from the interaction between plant genetics and environment ($\eta^2_{GxE}$) was relatively small across all trait and insect phenotypes. Results from sensitivity analyses remained largely consistent with these findings (Supplemental Fig. 1, means were slightly shifted and the confidence intervals increased in size).

*Question 2: Relative strength of environmental treatments*

My analyses revealed little environmental variation for levels of condensed tannins and salicinoids, and insect herbivore performance. I therefore did not attempt to further partition $\eta^2_E$ variation across environmental treatments for these traits. Instead, results for those traits across environments are presented in terms of plasticity ($\eta^2_E$ + $\eta^2_{GxE}$) in the next section. Here, I focus on environmental variation in plant growth and foliar nitrogen.

Different environmental treatments varied in the extent to which they contributed to phenotypic variation in the two most environmentally-responsive plant traits, growth and foliar nitrogen levels (Fig. 5). Both soil nutrients and pathogen (rust) inoculation

strongly influenced variation in plant growth, while changes in carbon dioxide, ozone, water (both drought and flooding), defoliation, and light all minimally influenced growth variation. In comparison, soil nutrients, competition, and light all strongly influenced variation in foliar nitrogen levels, while ozone and defoliation had small effects. Some environmental factors exhibited large $\eta^2_E$ variability across studies (*e.g.,* large confidence intervals) in their effect on plant growth and foliar nitrogen, including site characteristics, soil texture (clay and sand content) and temperature.

These results were sensitive, however, to the modeling approach used. Results from sensitivity analysis differed from these findings for growth and foliar nitrogen in varying light, competition, defoliation, rust (pathogen), water, and soil texture and nutrient environments (Supplemental Fig. 2). These differences are likely due to limited numbers of studies for particular environmental treatments (which were then affected by model parameters such as 'Study ID'). Comparing the results from the two modeling approaches, particular findings from each approach are supported by previous work. The relatively strong effects of light (Fig. 5), competition (Fig. 5), and water (sensitivity analysis, Supplemental Fig. 2) environments in shaping variation in growth and foliar nitrogen has been documented previously (Griffin et al. 1991, Donaldson et al. 2006a, Osier and Lindroth 2006, Hogg et al. 2008). Thus, the main results (Fig. 5) for *most* environmental treatments corresponded with previous research, while results from the sensitivity analysis for water and light effects on plant growth were also supported by previous findings.

*Question 3: Relative plasticity of traits*

Plant traits and insect performance varied in their plasticity (E + GxE) across environmental treatments (Fig. 6). Plant growth was most plastic across various study sites (locations) and with different water treatments and levels of soil nutrients. Foliar phenolics, condensed tannins, and nitrogen, as well as insect herbivore performance also exhibited increased plasticity across soil nutrient environments. In addition, both foliar nitrogen and insect performance were plastic in response to carbon dioxide treatments. All traits (growth, phytochemistry, and insect performance) exhibited low plasticity across ozone and defoliation treatments. Results from sensitivity analysis remained largely the same, except that insect herbivore performance was not as plastic (decrease of ~0.15) in response to changes in soil nutrients (Supplemental Fig. 3).

*Question 4: Comparison of insect herbivores*

Variation in insect herbivore performance that was shaped by plant genetics ($\eta^2_G$) differed across insect groups (order and specialization, Fig. 7 and Supplemental Fig. 4), depending on the model approach. Accounting for dependence between estimates from the same study increased the heterogeneity across studies (*i.e.*, larger confidence intervals, Fig. 7), and thus the confidence intervals largely overlapped. Yet, results from sensitivity analysis revealed significant differences across insect groups in the amount of variation in insect performance that was determined by plant genetics (Supplemental Fig. 4). Plant genetics had the strongest effects on variation in performance for lepidopteran (*e.g.,* gypsy moth, forest tent caterpillar, leaf mining species), coleopteran (leaf beetles), and dipteran (leaf galling flies) insects compared to hymenopteran (sawflies) and hemipteran (aphids) species. In addition, performance of generalist

insects (*e.g.,* gypsy moth, forest tent caterpillar) was shaped more by plant genetics than was the performance of specialist insects (*e.g.,* leaf-modifying insects).

**Discussion**

Intraspecific plant variation structures communities and ecosystems (Crutsinger et al. 2006, Hughes et al. 2008, Bolnick et al. 2011, Violle et al. 2012, Lamit et al. 2014, Koricheva and Hayes 2018). Yet, previous meta-analyses have either focused on intraspecific variation at the plant phenotype-level (Bolnick et al. 2011, Siefert et al. 2015) or neglected intraspecific variation altogether to focus on changes in plant trait means in response to environmental treatments (Koricheva et al. 1998, Valkama et al. 2007, Dios et al. 2016, Li et al. 2016). To quantify plant intraspecific variation across different environments, I partitioned plant phenotypic variation into its genetic, environmental, and GxE components. My findings reveal that (1) defense phytochemistry and insect performance were shaped more by plant genetics than were plant growth and foliar nitrogen, which were primarily environmentally-determined. (2) Soil nutrient, water, and pathogen environments were stronger than defoliation treatments in shaping plant phenotypic variation (growth and foliar chemistry). (3) Phenotypic plasticity varied substantially across both plant traits and environmental contexts, accounting for 8% to 58% of the trait variation. (4) Plant genetics played a relatively large role in shaping the variation in insect herbivore performance, and this role was potentially larger for generalist lepidopteran species than specialists and/or hemipteran species. These findings highlight the importance of intraspecific variation in shaping the evolvability and plasticity of traits and in structuring plant-insect interactions.

*Relative effects of G, E, and GxE across growth, phytochemistry, and insect performance*

My results revealed that the capacity for evolution (as indicated by genetic variation) differed across Salicaceae traits and was highest for defense compounds (salicinoids and condensed tannins). This finding corresponds well with a meta-analysis of heritability estimates for plant functional traits (Geber and Griffen 2003), which found that plant secondary chemistry was more heritable than plant growth and size metrics ($h^2$ = 0.43 and 0.16, respectively). Secondary metabolites mediate plant interactions with the environment and thus often undergo variable selection pressures across space and time, resulting in highly variable expression across plant genets (Moore et al. 2014, Lämke and Unsicker 2018). Levels of salicinoids and condensed tannins in Salicaceae species can vary up to 10-fold across genets in a common location (Förster et al. 2010, Boeckler et al. 2011, Lindroth and St. Clair 2013).

My results also showed that Salicaceae has a high capacity for evolution in response to interactions with insect herbivores. The extent to which plant genetic variation shaped insect herbivore performance was relatively high (~30%), and this effect can feed back to influence plant evolutionary trajectories for resistance or tolerance to insects. In addition, this capacity may vary with insect order and across specialist and generalist insect feeders (depending on the particular meta-analysis model). Salicaceae's capacity for evolution was highest for generalist lepidopteran herbivores, which were also the most studied group of insects in my dataset. Generalist lepidopterans often exhibit decreased fitness and performance on diets rich in chemical

defense compounds, especially salicinoids (Boeckler et al. 2011, Lindroth and St. Clair 2013). Since variation in Salicaceae chemical defense was largely shaped by plant genetics and these compounds determine generalist lepidopteran performance, salicinoids likely govern the plants' capacity to evolve resistance to these insects. This pattern does not necessarily hold, however, for other types of insects (*e.g.,* specialist leaf beetles) and across other plant taxa. A meta-analysis of 40 plant species from 19 families by Carmona et al. (2011) found that plant resistance to herbivores was shaped primarily by the plants' life-history and morphology traits, and secondarily by chemical defense traits.

*Relative effects of environmental factors and trait plasticity*

Plants respond to environmental change by both phenotypic plasticity and evolutionary adaptation (Franks et al. 2014, Hendry 2016). The magnitude of phenotypic plasticity within a plant population highlights the capacity for tolerance to particular environmental stressors. My findings revealed that plasticity varied substantially across both plant traits and environmental contexts, accounting for 8% to 58% of the trait variation. Plasticity was highest for plant growth, foliar nitrogen levels, and insect herbivore performance for plants grown in different sites and treatments of soil nutrients, water, and carbon dioxide. These findings correspond with meta-analyses (Koricheva et al. 1998, Li et al. 2016, Fabio and Smart 2018) of *Salix* and broader plant taxa for the effects of varying nutrient, carbon dioxide, and water environments on foliar nitrogen, growth, and insect performance. Plasticity was lowest for defense compounds (especially for plants grown with varying water levels) and was consistently low across

ozone and defoliation environments. The low plasticity of traits and insect performance across ozone environments correspond with meta-analyses by Valkama et al. (2007) and Koricheva et al. (1998), which found that increased ozone levels did not change particular plant traits (foliar nitrogen, tannins) and insect metrics (relative growth rate, survival, fecundity).

Some of my findings appear to contradict previous research. In particular, condensed tannins have exhibited large plasticity in some studies (Donaldson et al. 2006a, Osier and Lindroth 2006) and varying water treatments can significantly alter plant growth (Griffin et al. 1991, Hogg et al. 2008). Condensed tannins are plastic across varying competition and light environments (Donaldson et al. 2006a, Osier and Lindroth 2006), yet those treatments were rare in my meta-analysis dataset. Thus, the less plastic responses of condensed tannins in environments that were common in my analyses (*e.g.,* soil nutrients, enhanced $CO_2$, defoliation, etc.) drove the patterns in my results. In addition, I found that water treatments (both addition and removal) accounted for a minimal amount of the variation in plant growth ($\eta^2_E$, Fig. 5). Yet this result was mainly due to modeling parameters and the distinction between environmental effects ($\eta^2_E$) and plasticity ($\eta^2_E + \eta^2_{GxE}$). Results from the sensitivity analysis showed that water treatments accounted for roughly 20% of the variation in plant growth (Supplemental Fig. 2), while plasticity (E + GxE) accounted for 40% of growth variation (Fig. 6). Thus, when study-level heterogeneity was controlled (via the multilevel model structure) and genotype by environment interactions were ignored, the large effects of water on plant growth, which has been shown in many studies (Griffin et al. 1991, Hogg et al. 2008), was negated.

*Heterogeneity across studies*

Heterogeneity of effect sizes across studies is known to be high in ecological meta-analyses (Nakagawa et al. 2017). Even with my targeted study system, Salicaceae (*Salix* and *Populus*), my analyses exhibited very high heterogeneity across studies (> 80%, Supplemental Table 2). This result is likely due in part to the nature of heritability and the partitioning of trait variation, which is population and context-dependent (Falconer and Mackay 1996). In populations with more allelic diversity, genetics tends to play a stronger role in shaping phenotypic expression (Falconer and Mackay 1996). Conversely, in populations that experience larger environmental variation, environmental factors tend to play a stronger role in shaping phenotypic expression (Falconer and Mackay 1996). Interestingly, both the number of species and genets (which indicates allelic variation) within the Salicaceae populations and the ratio of number of species/genets to the number of levels of the environmental treatment (which indicated the extent of the allelic and environmental variation) were never selected as covariates in my meta-analysis models. This finding reveals that my results are robust to differences in population-level characteristics and likely represent general trends in the partitioning of variance across Salicaceae traits and environmental contexts.

*Future directions*

My quantitative review revealed a rather limited representation of factors important to the ecological success of Salicaceae in studies of genetic and

environmental variation. While many published studies investigated replicated common gardens across different locations, they overwhelmingly surveyed only growth and yield traits. Future studies should assess a larger array of plant traits as well as interactions with other species, such as insect herbivores. In addition, more controlled environmental manipulations are needed. Specifically, manipulations of plant biotic environment (*e.g.,* competition, defoliation, disease, etc.), light environment, and water treatments, with assessment of more than growth response, are needed.

Another need for future research is a greater emphasis on older plants. Many of the studies in my meta-analysis evaluated trees in their first or second year of growth; only nine out of 72 studies surveyed trees that were five years of age or older. A previous meta-analysis (Barton and Koricheva 2010) reported that plant defense and interactions with herbivores change with plant ontogeny. Indeed, foliar defense traits change markedly over plant developmental age in some (Donaldson et al. 2006b), but not all (Nissinen et al. 2018), Salicaceae.

For studies that evaluate Salicaceae-insect interactions, greater taxonomic breadth of insects is needed. Nearly half (6 out of 15) of the studies I analyzed focused on only two generalist lepidopteran species: gypsy moth (*Lymantria dispar dispar* L.) and forest tent caterpillar (*Malacosoma disstria* Hubn.). While these insects have environmental and economic importance (Roland 1993, Aukema et al. 2011), more experiments are needed that assess the performance of diverse insect guilds (*e.g.,* miners, gallers, rollers, suckers) and specialist insect species.

Finally, I recommend that additional meta-analyses, across different plant taxa, partition plant phenotypic variation into genetic, environmental, and GxE components.

Such information would help clarify whether similar patterns in the partitioning of phenotypic variation hold across plant families, groups (*e.g.,* angiosperms vs. gymnosperms, woody vs. herbaceous plants, crops vs. wild relatives, temperate vs. tropical plants), and defense strategies (*e.g.*, plants with nitrogen-containing defense compounds vs. carbon-based compounds, physical vs. chemical defense).

*Conclusions*

Deconstructing phenotypic variation into its genetic, environment, and genotype by environment (GxE) components can help enhance understanding of evolutionary trajectories and population responses to spatially and temporally variable environments. This quantitative review of intraspecific trait variation in Salicaceae reveals differences in the capacity for traits to evolve and to tolerate environmental stress. Chemical defense compounds exhibited a high capacity for adaptive evolution, while growth and foliar nitrogen exhibited strong plasticity in response to environmental treatments. I found that Salicaceae populations have a relatively strong capacity to adapt to insect herbivory via evolution of resistance or tolerance, and that this capacity may vary across groups of insects (*i.e.*, stronger evolvability in response to generalist compared with specialist insects). In addition, plasticity varied considerably across Salicaceae traits and environments. These findings highlight the importance of quantifying the genetic, environmental and GxE components of plant intraspecific variation in the context of research in evolutionary ecology.

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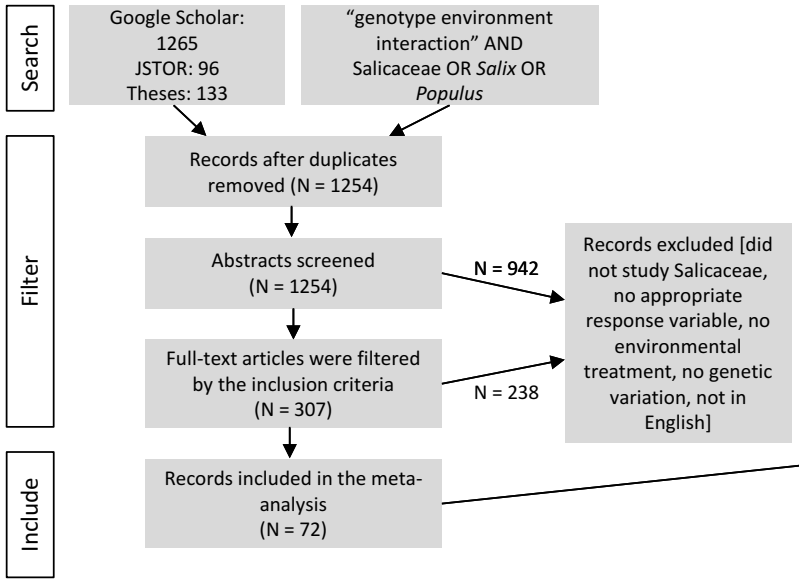
**Table 1.** Summary of the multilevel mixed models for meta-analyses. Question corresponds to the four study questions posed in the Introduction. Within the models, "factor" designates categorical variables, "random" designates the random effect of study ID to control for non-independence of effects within a study (*i.e.,* multilevel structure), and "y" is the logit-transformed generalized eta squared ($\eta^2$) variation.

| Question | Data set | Model | Multilevel variance component | Number of studies | N | Test for residual heterogeneity | | | Test of moderators | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | QE | df | P | QM | df | P |
| 1 | Condensed tannins | y = factor(G/E/GxE) + random(StudyID) | 0.640 | 25 | 111 | 1857.99 | 108 | <0.001 | 1421.48 | 3 | <0.001 |
| | Phenolic glycosides | | 0.391 | 22 | 183 | 2771.38 | 180 | <0.001 | 4189.81 | 3 | <0.001 |
| | Tree growth/size | y = factor(G/E/GxE) + factor(EnvironmentalTreatment) + random(StudyID) | 0.863 | 53 | 513 | 9306.75 | 493 | <0.001 | 1655.68 | 14 | <0.001 |
| | Foliar nitrogen | | 1.724 | 21 | 96 | 1731.95 | 86 | <0.001 | 418.85 | 10 | <0.001 |
| | Insect herbivore performance | | 0.985 | 15 | 216 | 4194.54 | 207 | <0.001 | 3077.61 | 9 | <0.001 |
| 2 | Tree growth/size | y = factor(EnvironmentalTreatment) + random(StudyID) | 8.241 | 51 | 169 | 3395.04 | 152 | <0.001 | 817.44 | 11 | <0.001 |
| | Foliar nitrogen | | 3.611 | 21 | 32 | 416.14 | 24 | <0.001 | 235.60 | 8 | <0.001 |
| 3 | Site characteristics | y = factor(PlantTrait) + random(StudyID) | 1.037 | 22 | 72 | 535.04 | 69 | <0.001 | 11.34 | 3 | 0.01 |
| | Soil nutrients | | 1.385 | 28 | 114 | 1289.84 | 105 | <0.001 | 415.24 | 6 | <0.001 |
| | Water | | 2.400 | 7 | 22 | 98.93 | 18 | <0.001 | 8.82 | 4 | 0.066 |
| | Ozone | | 1.986 | 5 | 26 | 209.53 | 21 | <0.001 | 24.50 | 5 | 0.002 |
| | Defoliation | | 0.953 | 8 | 36 | 215.24 | 31 | <0.001 | 87.70 | 5 | <0.001 |
| | Carbon dioxide | y = factor(PlantTrait) + TreeAge + random(StudyID) | 0.740 | 10 | 40 | 205.41 | 34 | <0.001 | 92.54 | 6 | <0.001 |
| 4 | Insect herbivore performance | y = factor(InsectOrder) + random(StudyID) | 1.181 | 14 | 60 | 525.59 | 55 | <0.001 | 9.54 | 5 | 0.089 |
| | | y = factor(InsectSpecialistGeneralist) + random(StudyID) | 0.417 | 15 | 68 | 610.57 | 66 | <0.001 | 13.25 | 2 | 0.001 |

N = number of generalized eta squared estimates in the response variable for a given model

**Figure 1.** Meta-analysis approach to searching for records for the database and filtering those records for inclusion in the analyses. Number of records (publications) are denoted as the sample size (N). Number of sets of estimates (generalized eta squared, $\eta^2$) of the amount of phenotypic variation that is explained by plant genotype (G), environmental treatment (E), and their interaction (GxE) are shown in the table. The table includes information for both the 72 included records and the 50 excluded records that did not report the appropriate statistics to derive $\eta^2$.




| | | Included | Excluded |
|---|---|---|---|
| Subject | *Populus* | 360 | 124 |
| | *Salix* | 114 | 51 |
| Response | Growth | 179 | 101 |
| | Condensed tannins | 53 | 4 |
| | Salicinoids | 67 | 14 |
| | Nitrogen | 48 | 11 |
| | Insect herbivore | 110 | 14 |
| Environment | Field site | 83 | 26 |
| | Nutrients | 155 | 31 |
| | Soil texture (e.g, sand) | 17 | 8 |
| | Salt | 2 | 3 |
| | Temperature | 16 | 0 |
| | $CO_2$ | 53 | 16 |
| | $O_3$ | 39 | 48 |
| | Water | 22 | 35 |
| | Defoliation | 56 | 3 |
| | Pathogen inoculation | 9 | 2 |
| | Competition | 13 | 3 |
| | Light | 23 | 7 |

**Figure 2.** Contour-enhanced funnel plots of (A) the observed logit-transformed generalized eta squared ($\eta^2$) values, and model residuals for the (B) growth data, (C) salicinoid data, (D) condensed tannin data, (E) foliar nitrogen data, and (F) insect herbivore performance data. The models are shown in Table 1 under question one. Each dark circle is either an observed effect (for panel A) or a residual (panels B-F). Each white circle (panel A) is an estimated effect from a "missing" study (unpublished), derived from Duval and Tweedie's (2000) 'trim and fill' method. The background shading in the plots represents the significance of the study estimates (white $P > 0.10$, light gray within the funnel $0.10 > P > 0.05$, dark gray within the funnel $0.05 > P > 0.01$, outside the funnel $P < 0.01$). Estimates missing from non-significant portions of the funnel indicate potential underlying publication bias. Conversely, if estimates are missing from significant portions of the figure, this indicates that other factors (*e.g.,* study quality) are causing the asymmetry in the plot.

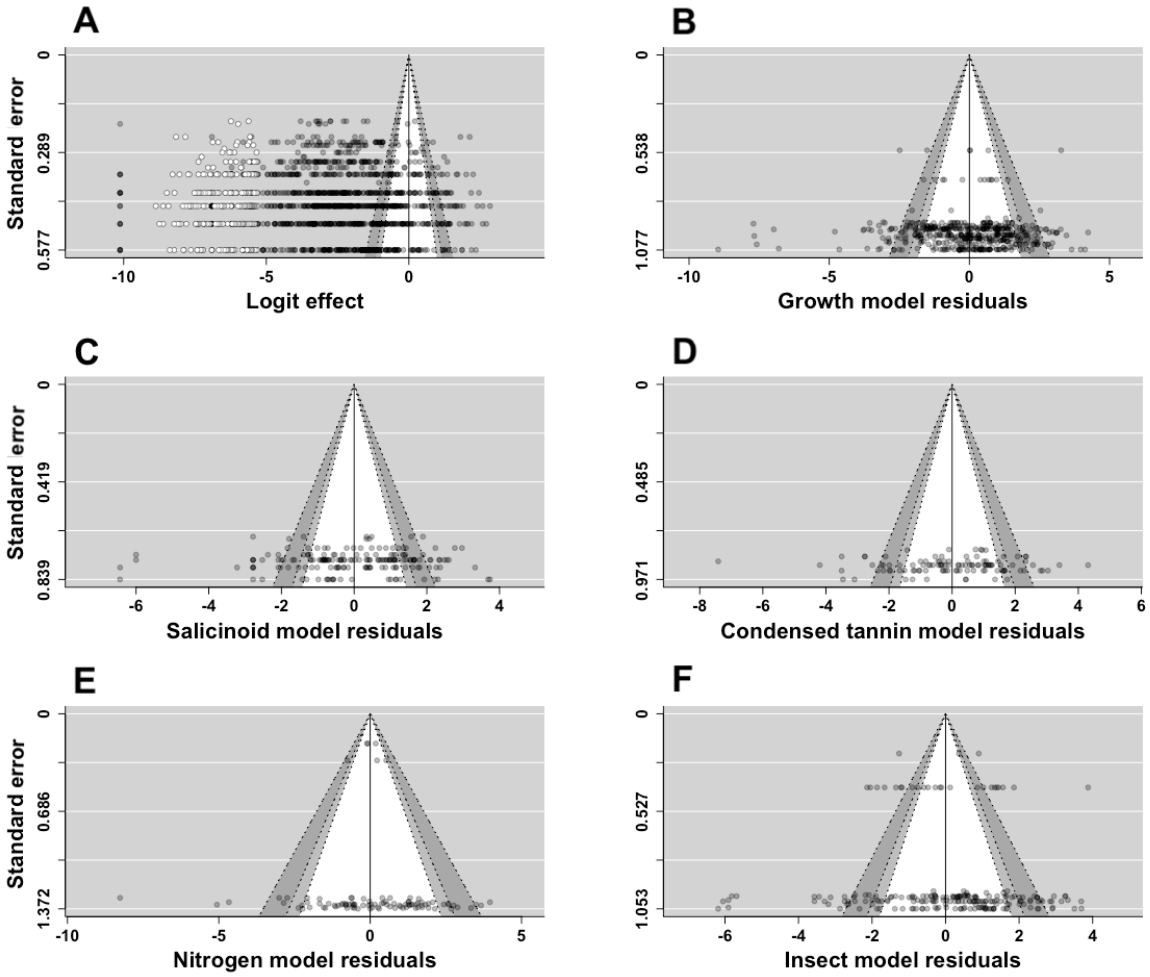

A
Standard error
0
0.289
0.577
-10
-5
0
Logit effect
B
0
0.538
1.077
-10
-5
0
5
Growth model residuals
C
Standard error
0
0.419
0.839
-6
-4
-2
0
2
4
Salicinoid model residuals
D
0
0.485
0.971
-8
-6
-4
-2
0
2
4
6
Condensed tannin model residuals
E
Standard error
0
0.686
1.372
-10
-5
0
5
Nitrogen model residuals
F
0
0.527
1.053
-6
-4
-2
0
2
4
Insect model residuals

**Figure 3.** Scatterplot of publication year and logit-transformed generalized eta squared ($\eta^2$) estimates for plant genetic variation (G). Each point is a genetic variation estimate from a particular study and the size of the point corresponds with the number of study replicates (*i.e.,* an estimate of study precision). The line is the predicted average effect (logit-transformed $\eta^2$) as a function of publication year with 95% confidence intervals (dashed lines).

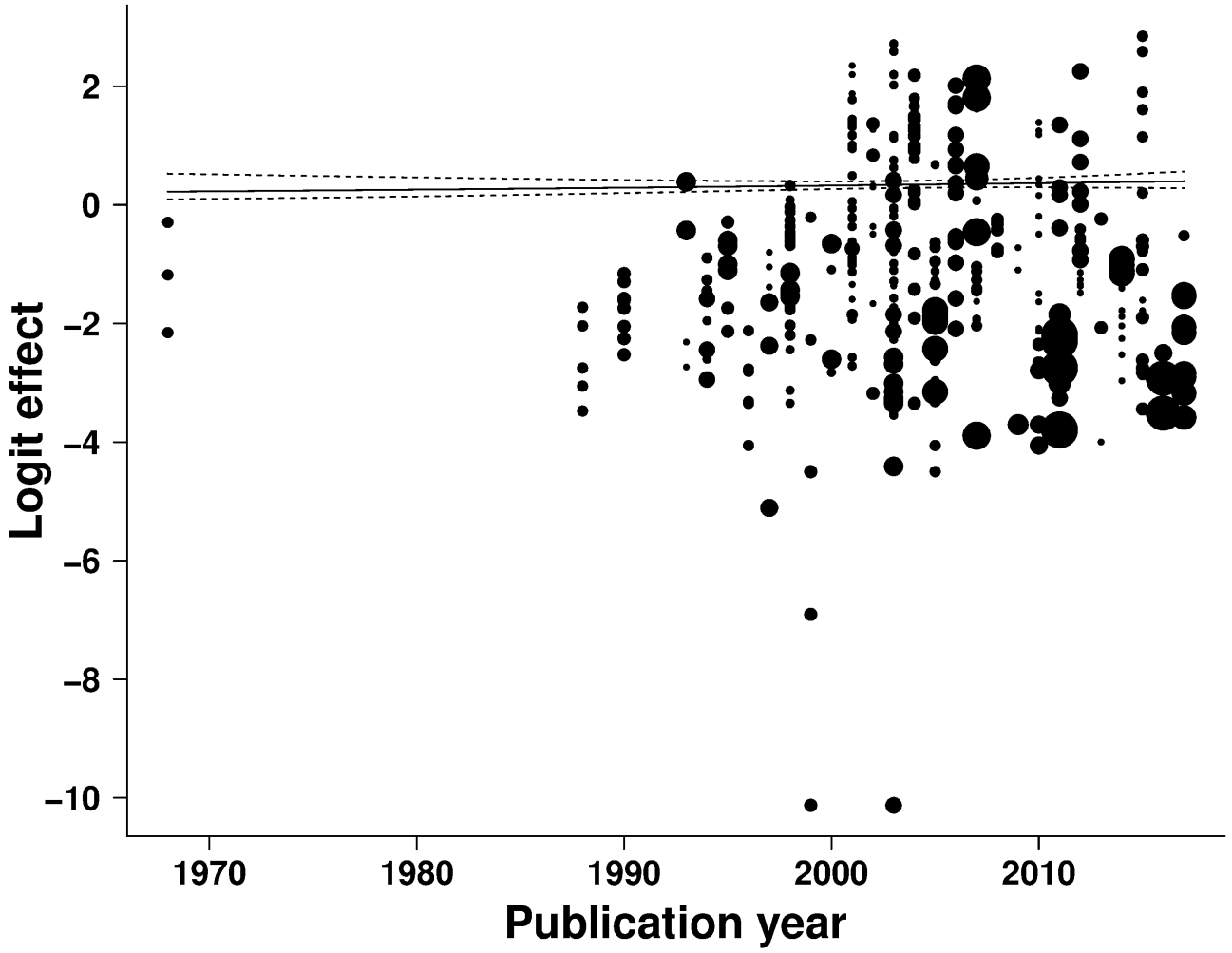

Logit effect
2
0
−2
−4
−6
−8
−10
1970
1980
1990
2000
2010
Publication year

**Figure 4.** Forest plot of the amount of phenotypic variation (generalized eta squared, $\eta^2$) that is explained by plant genotype (G), environmental treatment (E) and their interaction (GxE) for plant growth, foliar levels of condensed tannins, salicinoids, and nitrogen, and insect herbivore performance. Symbols represent the generalized eta squared ($\eta^2$) *variation* estimates, which were derived from models in Table 1 (under Question 1) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other. N is the number of $\eta^2$ estimates for each factor (G, E, and GxE) for a trait.

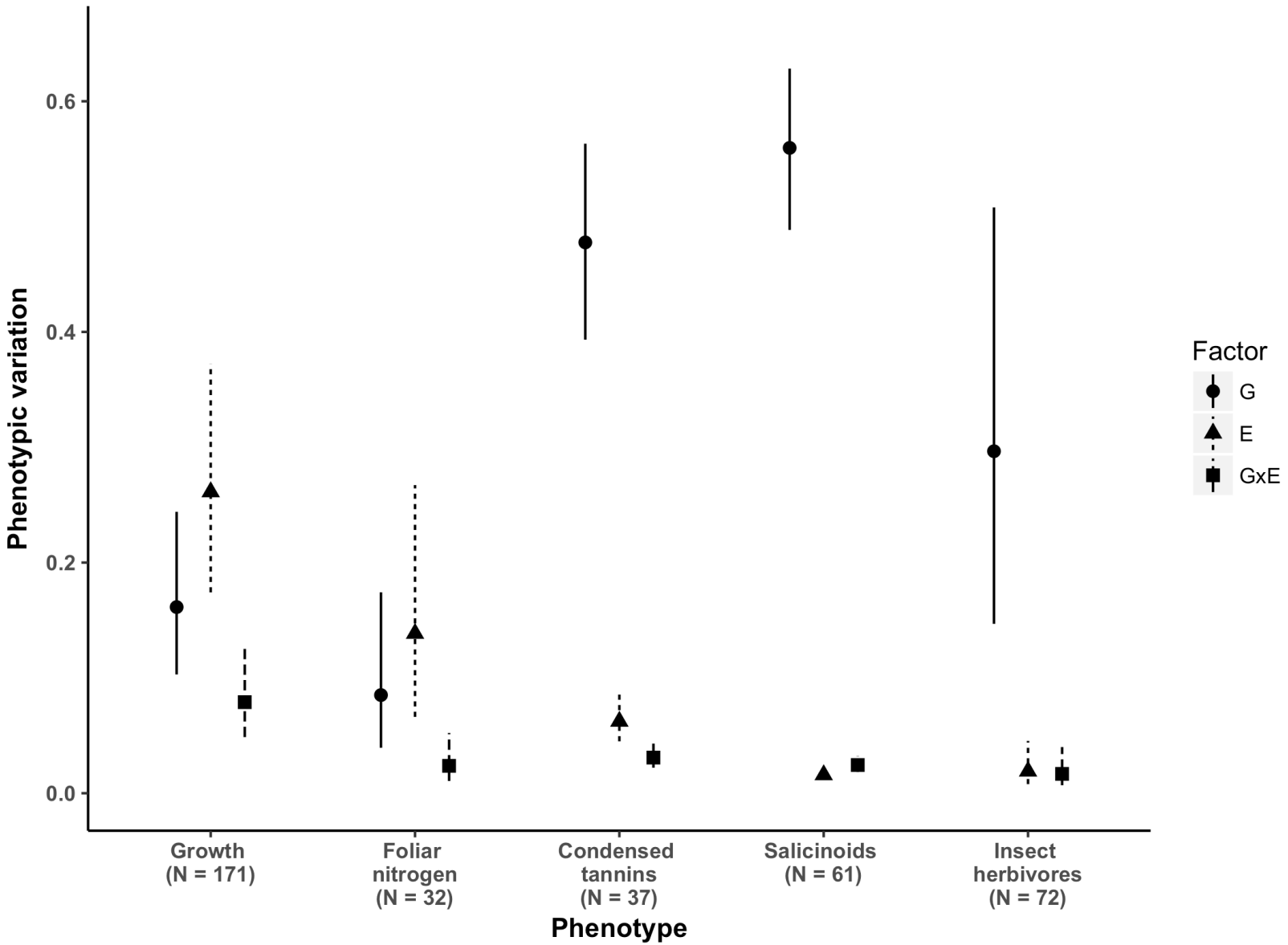

Phenotypic variation
0.6
0.4
0.2
0.0
Growth
(N = 171)
Foliar
nitrogen
(N = 32)
Condensed
tannins
(N = 37)
Salicinoids
(N = 61)
Insect
herbivores
(N = 72)
Phenotype
Factor
G
E
GxE

**Figure 5.** Forest plot of the amount of phenotypic variation that is explained by various environmental treatments (E) for both plant growth and foliar nitrogen levels. Symbols represent the generalized eta squared ($\eta^2$) *variation* estimates, which were derived from models in Table 1 (under Question 2) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other. The number above each confidence interval indicates the number of generalized eta squared estimates in each category.

Environmental variation
0.00
0.25
0.50
0.75
1.00
1
63
12
39
17
2
1
16
1
5
5
10
3
4
7
6
4
1
4
Site characteristics
Nutrients
Soil texture
Light
Water
Temperature
$CO_2$
$O_3$
Defoliation
Rust
Competiton
Environment
Trait
Foliar nitrogen
Growth

**Figure 6.** Forest plot of the amount of phenotypic variation that is explained by trait plasticity (E + GxE) under various environmental treatments for plant growth, foliar levels of condensed tannins, salicinoids, other phenolics, and nitrogen, and insect herbivore performance. Symbols represent the generalized eta squared ($\eta^2$) *variation* estimates, which were derived from models in Table 1 (under Question 3) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other. The number above each confidence interval indicates the number of generalized eta squared estimates in each category.

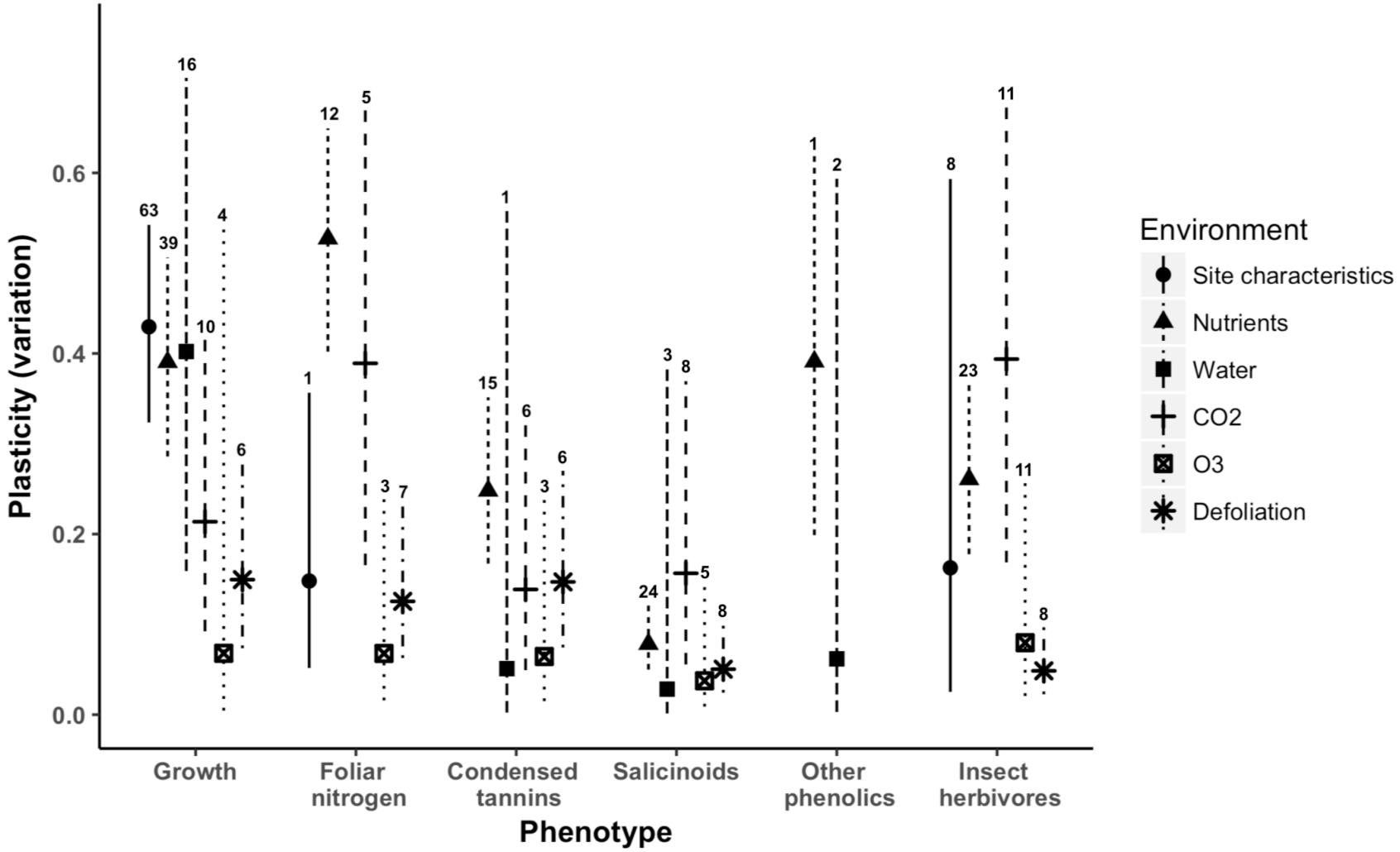

Plasticity (variation)
0.0
0.2
0.4
0.6
63
39
16
10
4
6
1
12
5
3
7
15
1
6
3
6
24
3
8
5
8
1
2
8
23
11
11
8
Growth
Foliar nitrogen
Condensed tannins
Salicinoids
Other phenolics
Insect herbivores
Phenotype
Environment
Site characteristics
Nutrients
Water
CO2
O3
Defoliation

**Figure 7.** Forest plot of the amount of variation in insect herbivore performance that is explained by plant genotype (G) for different groups of insects (orders, generalized vs. specialized insects). Symbols represent the generalized eta squared ($\eta^2$) *variation* estimates, which were derived from models in Table 1 (under Question 4) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other. The number above each confidence interval indicates the number of generalized eta squared estimates in each category.

Generalized eta squared
0.00
0.25
0.50
0.75
1.00
10
2
3
4
41
40
28
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Generalist
Specialist
Insect

**Supplemental Table 1.** Reference list for the 72 included studies in this Salicaceae meta-analysis. Included studies met particular criteria, outlined in Figure 1.

| # | Study citation |
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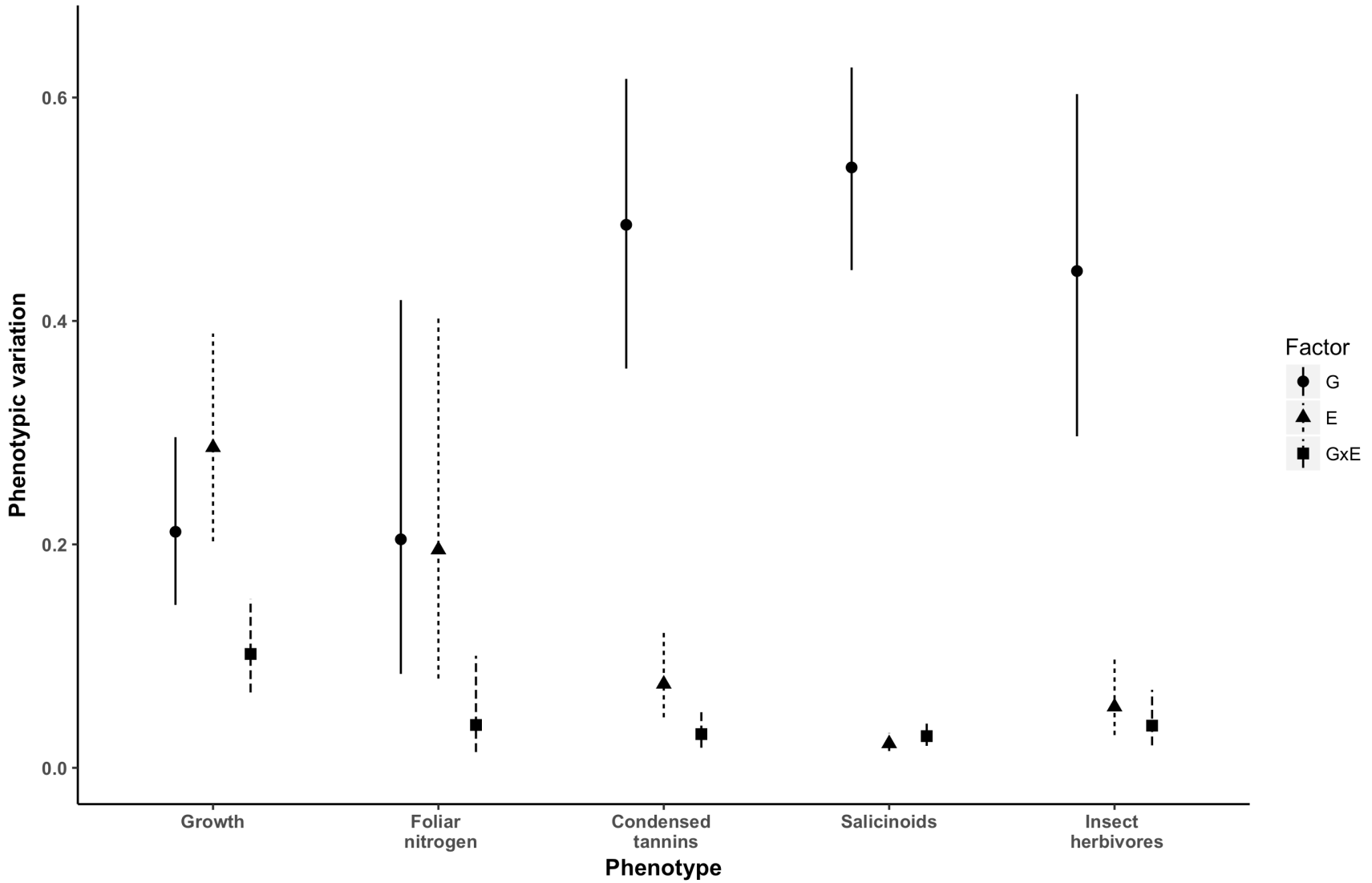
**Supplemental Table 2.** Summary of the sensitivity analysis: meta-analysis models without influential effects (N = 14) and without multilevel structure that accounts for non-independence among effects within a study. Question corresponds to the four study questions posed in the introduction. Within the models, "factor" designates categorical variables, and "y" is the logit-transformed generalized eta squared variation.

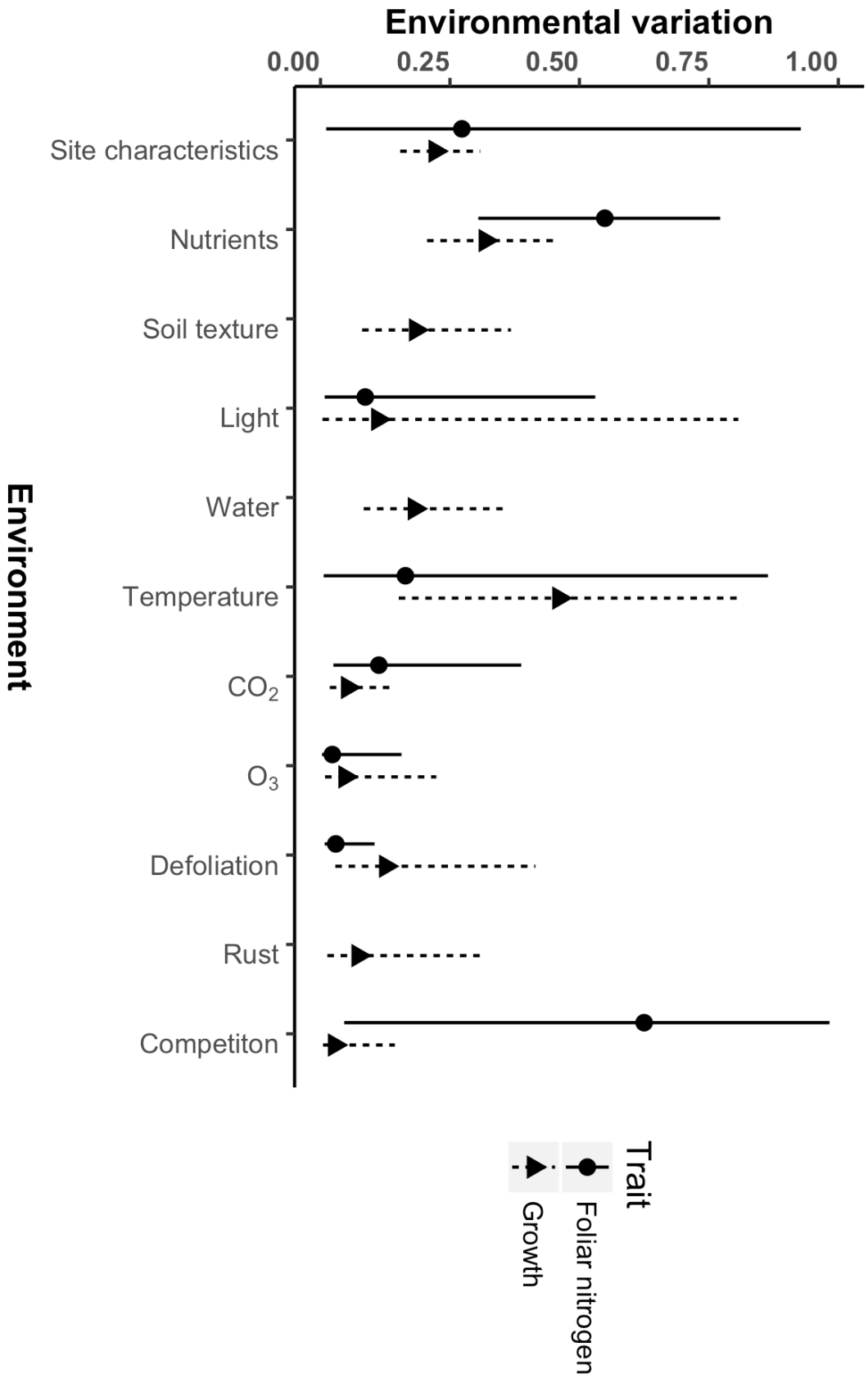
| Question | Data set | Model | Number of studies | N | $I^2$ (%) | Test for residual heterogeneity | | | Test of moderators | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | QE | df | P | QM | df | P |
| 1 | Condensed tannins | y = factor(G/E/GxE) | 25 | 110 | 93.19 | 1515.40 | 107 | <0.001 | 247.61 | 3 | <0.001 |
| | Phenolic glycosides | | 22 | 179 | 91.44 | 2063.27 | 176 | <0.001 | 737.87 | 3 | <0.001 |
| | Tree growth/size | y = factor(G/E/GxE) + factor(EnvironmentalTreatment) | 53 | 503 | 93.51 | 7057.00 | 489 | <0.001 | 1003.93 | 14 | <0.001 |
| | Foliar nitrogen | | 21 | 95 | 93.94 | 1333.71 | 85 | <0.001 | 170.09 | 10 | <0.001 |
| | Insect herbivore performance | | 15 | 210 | 93.27 | 2864.51 | 201 | <0.001 | 616.00 | 9 | <0.001 |
| 2 | Tree growth/size | y = factor(EnvironmentalTreatment) | 51 | 161 | 95.77 | 2890.34 | 150 | <0.001 | 123.97 | 11 | <0.001 |
| | Foliar nitrogen | | 21 | 32 | 94.12 | 416.14 | 24 | <0.001 | 49.70 | 8 | <0.001 |
| 3 | Site characteristics | y = factor(PlantTrait) + random(StudyID) | 22 | 72 | 87.99 | 535.04 | 69 | <0.001 | 29.78 | 3 | <0.001 |
| | Soil nutrients | | 28 | 111 | 91.80 | 1289.84 | 105 | <0.001 | 119.78 | 6 | <0.001 |
| | Water | | 7 | 22 | 84.01 | 98.93 | 18 | <0.001 | 46.51 | 4 | <0.001 |
| | Ozone | | 5 | 26 | 91.20 | 209.53 | 21 | <0.001 | 58.54 | 5 | <0.001 |
| | Defoliation | | 8 | 36 | 85.57 | 215.24 | 31 | <0.001 | 190.20 | 5 | <0.001 |
| | Carbon dioxide | y = factor(PlantTrait) + TreeAge | 10 | 40 | 83.49 | 205.41 | 34 | <0.001 | 161.91 | 6 | <0.001 |
| 4 | Insect herbivore performance | y = factor(InsectOrder) | 14 | 60 | 90.69 | 525.59 | 55 | <0.001 | 13.91 | 5 | 0.016 |
| | | y = factor(InsectSpecialistGeneralist) | 15 | 68 | 90.16 | 610.57 | 66 | <0.001 | 24.15 | 2 | <0.001 |

N = number of generalized eta squared estimates in the response variable for a given model
$I^2$ = heterogeneity statistic

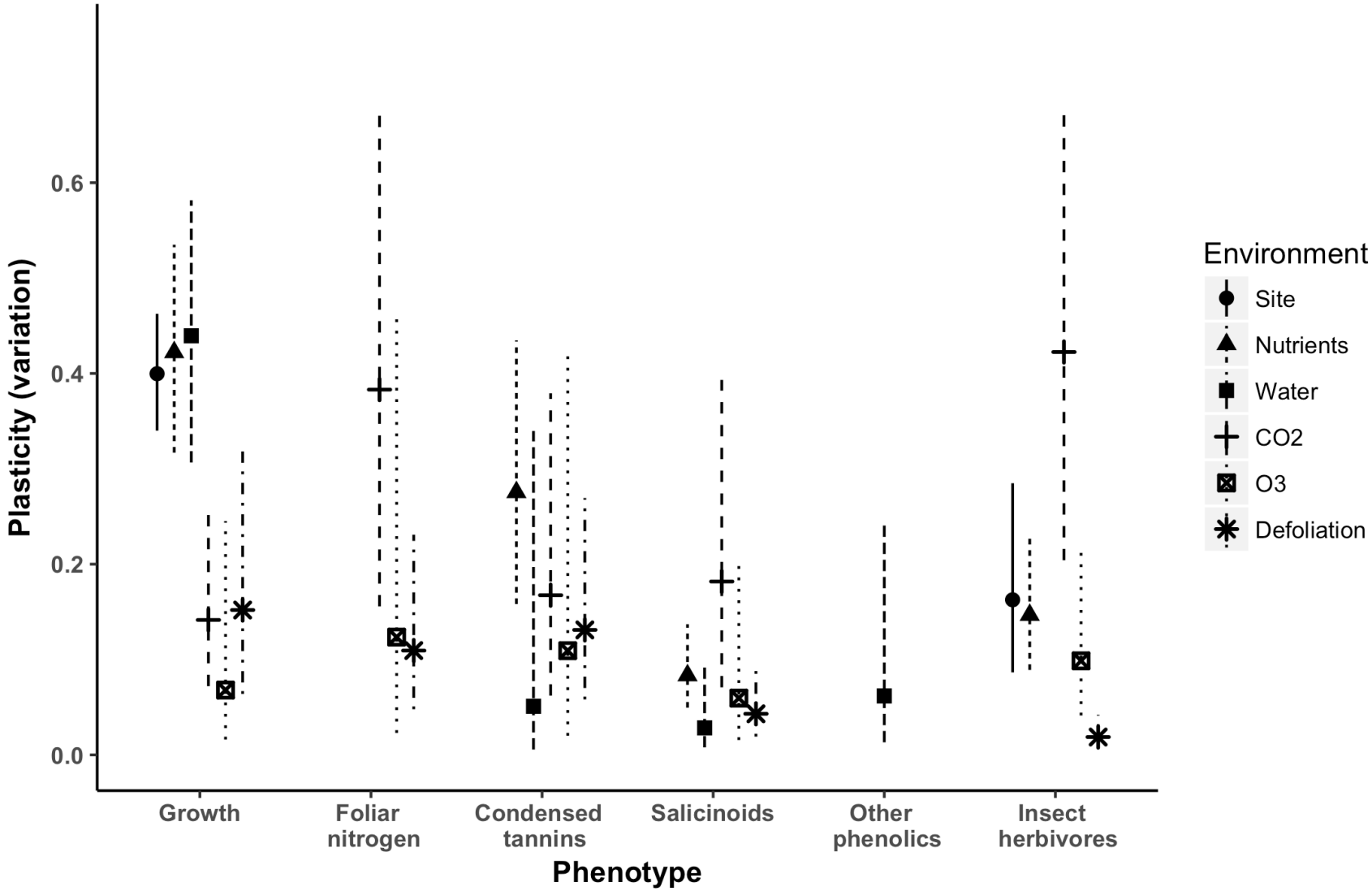
**Supplemental Figure 1.** Forest plot of the amount of phenotypic variation that is explained by plant genotype (G), environmental treatment (E) and their interaction (GxE) for plant growth, foliar levels of condensed tannins, salicinoid, and nitrogen, and insect herbivore performance. Symbols represent the generalized eta squared *variation* estimates, which were derived from sensitivity analysis models in Supplemental Table 1 (under Question 1) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other.


Phenotypic variation
0.6
0.4
0.2
0.0
Growth
Foliar nitrogen
Condensed tannins
Salicinoids
Insect herbivores
Phenotype
Factor
G
E
GxE

**Supplemental Figure 2.** Forest plot of the amount of phenotypic variation that is explained by various environmental treatments (E) for both plant growth and foliar nitrogen levels. Symbols represent the generalized eta squared *variation* estimates, which were derived from sensitivity analysis models in Supplemental Table 1 (under Question 2) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other.


Environmental variation
0.00
0.25
0.50
0.75
1.00
Environment
Site characteristics
Nutrients
Soil texture
Light
Water
Temperature
$CO_2$
$O_3$
Defoliation
Rust
Competiton
Trait
Foliar nitrogen
Growth

**Supplemental Figure 3.** Forest plot of the amount of phenotypic variation that is explained by trait plasticity (E + GxE) under various environmental treatments for plant growth, foliar levels of condensed tannins, salicinoids, other phenolics, and nitrogen, and insect herbivore performance. Symbols represent the generalized eta squared *variation* estimates, which were derived from sensitivity analysis models in Supplemental Table 1 (under Question 3) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other.


Plasticity (variation)
0.0
0.2
0.4
0.6
Growth
Foliar nitrogen
Condensed tannins
Salicinoids
Other phenolics
Insect herbivores
Phenotype
Environment
Site
Nutrients
Water
CO2
O3
Defoliation

**Supplemental Figure 4.** Forest plot of the amount of variation in insect herbivore performance that is explained by plant genotype (G) for different groups of insects (orders, generalized vs. specialized insects). Symbols represent the generalized eta squared *variation* estimates, which were derived from sensitivity analysis models in Supplemental Table 1 (under Question 4) and are shown with 95% confidence limits. Variation estimates with non-overlapping confidence intervals are significantly different from each other.

Generalized eta squared
0.00
0.25
0.50
0.75
1.00
Coleoptera
Diptera
Hemiptera
Hymenoptera
Lepidoptera
Generalist
Specialist
Insect

# Chapter Two

## Genotypic variation in plant traits shapes herbivorous insect and ant communities on a foundation tree species

Chapter Two is currently in review for PLOS ONE.

## Introduction

Community genetics is an emerging field of science that aims to link community ecology with evolutionary biology. Because genetics of host organisms can shape associated communities (Whitham et al. 2008), such communities can be viewed as extended phenotypes of the host. Accumulating studies, primarily in plant-insect systems (*e.g.*, Wimp et al. 2005, Johnson 2008, Zytynska et al. 2011, Barbour et al. 2015), have consistently supported the concept. Further progress in this field, however, has been constrained by limited information about the heritability of associated communities, the identification of core interactions within these communities, and the specific mechanisms that link plant genetics to associated communities.

Estimates of community heritability vary widely in the literature, from 0.00 for the diversity and composition of endophytes on *Populus angustifolia* (Lamit et al. 2014) to 0.81 for the abundance of galling insects on *Populus tremula* (Bernhardsson et al. 2013). To advance the field, we need to better understand the factors that underlie and constrain heritability estimates and identify which aspects of the community are most heritable. For instance, how does an organism's feeding mode (*e.g.,* galling vs. mining vs. free-feeding) influence heritability? Is there a relationship between community heritability and the heritability of plant traits that shape these communities? Do foundation or keystone community members have higher heritability estimates than other community members?

In addition, how these communities are structured (*e.g.,* relationships between organisms within the community) across plant genotypes remains poorly understood. The "interacting foundation species hypothesis" posits that relationships between

foundation species, such as the host plant and another ecologically-important species (*e.g.,* galling aphids), can shape the larger community (*e.g,* insect community, Keith et al. 2010, Lamit et al. 2015). Yet, these core interactions among foundation species are not always readily identified and may not exist in particular systems. Some studies (Bernhardsson et al. 2013, Barbour et al. 2015) investigate insect community structure across plant genotypes have found little evidence for significant relationships among insect species. This discrepancy across studies could be a product of differences in the scope of the community that is surveyed (*e.g.,* arthropods vs. only insect herbivores) and/or differences in the focal plant species (*i.e.,* most studies that support the "interacting foundation species hypothesis" have investigated communities on hybrid systems of poplars).

An additional criticism of community genetics is that community heritability values alone cannot distinguish whether associated communities are shaped more from underlying host genetics or environmental interactions (*e.g.,* microhabitat, competition among community members). Here, I compare "phenotype-based species interactions" (insect abundances for each plant) and "genotype-based species interactions" (insect abundances averaged by plant genet) between insect functional groups to (1) identify the key relationships among insect groups that underlie community heritability (*i.e.,* interacting foundation species) and (2) discern whether these key relationships are driven more by tree genotype or phenotype. In this comparison, phenotype-based species interactions quantify the correlation between two insect species (or functional groups) due to both environmental interactions and the underlying genetics of the host

tree, while genotype-based species interactions quantify the correlation between two insect species (or functional groups) that is a result of only the host tree genetics.

Although particular plant traits have been shown to function as mechanistic links between plant genetics and associated insect community structure (Bangert et al. 2006, Wimp et al. 2007), little is known about which traits are most important in shaping community structure (Crutsinger 2015). Of the twelve community genetic studies that have explored the effects of plant traits on insects, eight (Bangert et al. 2006, 2008, Wimp et al. 2007, Gosney et al. 2014, 2017, Crutsinger et al. 2014, Glassmire et al. 2016) have surveyed a limited set of traits, typically including only phytochemistry. In addition, few studies (Johnson and Agrawal 2005, Barbour et al. 2015, Glassmire et al. 2016) investigate how these traits influence individual species and/or groups of insects within the community. Thus, questions such as “are leaf-modifying insects more or less influenced by foliar chemistry compared with free-feeding insects?” remain unresolved.

To address these gaps, I explored herbivorous insect and ant communities on a diverse population of young trembling aspen (*Populus tremuloides)* grown in a newly established common garden. I investigated the heritability of insect communities and relationships among common insect functional groups (gallers, rollers, miners, free feeders, aphids, and ants). In addition, I evaluated how a wide array of tree traits (*i.e.,* morphology, phenology, phytochemistry, extra-floral nectaries) shape insect communities and influence individual species.

Aspen provides an ideal system for studying community genetics. It has available genetic resources (Street and Tsai 2010) and suitable population qualities (*e.g.,* low structure; Ingvarsson 2010) to identify genes underlying extended phenotypes (*e.g.,*

insect communities). Aspen is a foundation species, in that it plays important roles in structuring ecosystems (Ellison et al. 2005, Madritch et al. 2009), and it is the most widely distributed and genetically diverse tree species in North America (Mitton and Grant 1996). The insect community associated with aspen is appropriate for this research because the number of common insects is manageable for identification (*i.e.,* 19 common species, Table 1, Hillstrom and Lindroth 2008).

This research was structured to address four questions: (1) Which aspects of insect community structure (*e.g.,* richness, abundance) are heritable? (2) Are there strong genotype-based species interactions (indicative of interacting foundation species) and if so, what is their relative importance compared with phenotype-based species interactions for structuring insect communities? (3) Which plant traits are most important in structuring associated insect communities? (4) How do plant traits influence the presence and abundance of individual species and functional groups within the insect community?

## Methods

### *WisAsp common garden*

The Lindroth Lab Group established an aspen common garden (WisAsp) in 2010 with 392 replicated genets that were collected from throughout Wisconsin and propagated from rootstock. The aspen saplings were planted in a completely randomized block design with four blocks and a perimeter of non-experimental trees. The trees were planted with 2.5 x 2.5m spacing and $1m^2$ weed mats to reduce weedy growth next to the tree's stem. The site has a Joy silt loam soil type and was previously

used as a grass-alfalfa hayfield. I did not add any soil supplements. To help establish the newly planted trees, they were watered (approximately weekly) in the summer of 2010 and the site was routinely mowed. The garden was enclosed with a 2.4m tall electrified fence to prevent damage by deer.

Due to vole herbivory, I lost 399 and 334 trees in 2010 and 2011, respectively. These trees were then replaced with new genets, resulting in a total of 445 genets (3.56 replicates/genet on average) with three distinct age classes. For the research reported here, I included only the trees that were originally planted in 2010, to eliminate differences in tree age (328 genets, 2.95 replicates/genet on average).

*Tree traits*

I measured multiple ecologically-important tree traits, including leaf area, tree size, phytochemistry, extra-floral nectary (EFN) density, and timing of bud set and bud break. I collected measurements for all traits except EFNs in both 2014 and 2015; EFN density was recorded only in 2014.

To measure leaf area, I haphazardly collected 15-25 leaves from each tree in July (2014-15) and stored them on ice in the field. I brought the leaves to the laboratory to scan them for leaf area using a flatbed LiCor scanner (Version 3100, Lincoln, NE). I then vacuum-dried, weighed, ball-mill ground, and stored the leaves at -20$^{o}$C until used for chemical analyses.

I first scanned the ground leaf samples to capture near infrared spectroscopy (NIRS) information (FOSS NIRS Systems 6500 spectrometer). I then analyzed a subset (200-300) of leaf samples via standard wet chemistry techniques. These data were

used to fit a partial least squares generalized linear model for estimating chemical components of remaining samples (~1424) by NIR spectra (NIRS3 software [Version 3.10. Infrasoft, Silver Spring, MA]). To analyze samples via wet chemistry methods, I used combustion gas chromatography for C:N analysis (Thermo Flash EA1112 elemental analyzer [Thermo Finnigan, Milan, Italy]), the HCl-butanol spectrophotometric method (Porter et al. 1986) for condensed tannins (with purified *P. tremuloides* condensed tannin [(Hagerman and Butler 1980)] as reference standard), and ultra-high pressure liquid chromatography (Waters ACQUITY iClass UPLC/MS system, Milford, MA, USA) with mass spectrometry for phenolic glycosides (with purified salicin, salicortin, tremuloidin, and tremulacin as reference standards; methods adapted from Abreu et al. [2011] and Rubert-Nason et al. [2014]).

To measure tree size, I surveyed the basal diameters (10 cm above ground) and heights (to the base of the apical meristem) of the trees after each growing season. To calculate tree volume, I used $basal\ diameter^2 \times height$ (Stevens et al. 2007). Since insect communities were surveyed in July, I calculated the average tree volume (averaged across fall measurements before and after an insect survey) to better approximate tree size at this time in the growing season.

To measure the density of EFNs, I haphazardly collected 12 leaves from each tree in August 2014 and digitally scanned the upper surface of the leaves. I then visually scored the number of EFNs that were present on each leaf. Scores varied from zero to four.

Both bud break and bud set were recorded by surveying the trees with a preset phenological scale. For bud break, I used a 5-point scale (1: set dormant bud, 2: green

bud, 3: broken bud, 4: leaves flushed but curled, 5: leaves fully expanded) and recorded the most advanced bud on a tree, and for bud set I used a 2-point scale (set vs. growing bud) and measured the apical bud on the main leader. I surveyed each tree every 2-3 days until at least 95% of the trees had advanced to the end of the survey scale.

*Insect communities*

I visually surveyed herbivorous insects and ants during the height of the insect season in mid-July (2014-15). I first assessed the arthropod communities at WisAsp by producing species area curves standardized by time to ensure that the visual surveys were conducted in a manner that captured the entire community. Based on these curves, I designated time intervals in which to survey each tree. I surveyed smaller trees for one time unit (3min) and larger trees were surveyed for additional time units (with the number of units depending on tree size), and thus insect counts across trees could be directly compared as the number of insects per species observed per minute. In 2014, I surveyed the entire tree canopy of each tree. In 2015, sizes of some trees precluded surveys of the entire canopy, so I surveyed a large subset of the canopy (for large trees) or the entire canopy (for small trees). To ensure that my survey effort was not skewed to particular regions of the canopy, I first divided each tree into low, middle and upper canopy sections. I then spent equal amounts of time surveying each section (*e.g.,* for small trees, the 3min time unit was divided so that 1min was spent surveying each canopy section). Insect abundances were then divided by survey time for each tree (*i.e*., number of insects per species per minute) so that insect counts across trees of different sizes and survey times could be directly comparable.

I recorded every insect observed and all insect-inflicted damage (*e.g.,* vacant leaf rolls and mines). Survey times were interrupted to collect, identify, and record insects. Insect specimens are kept in a voucher collection in the UW-Madison Wisconsin Research Insect Collection, and all common insects are identified to species and rare insects to morpho-species (Family-level). See supplemental material for a list of both the resources used to identify insect specimens (Supplemental Table 1) and the insect species and morpho-species observed in this study (Supplemental Table 2).

*Statistical analyses*

For statistical analyses, the insect community data were processed according to standard practices (McCune and Grace 2002). For instance, the insect data were standardized by survey time (*e.g.,* number of insects per species per minute) and rare insects (recorded on <5% of the surveyed trees; Supplemental Table 3) were omitted to reduce statistical noise. The data were then square root transformed to reduce the influence of very abundant outlier species (*e.g.,* aphids). Because I was interested in overall differences in total insect abundances across surveyed trees, I did not relativize the insect data (*e.g.,* by total insects per tree or site). I assessed insect communities with several metrics, including species richness, Shannon index, total abundance, and community stability.

To calculate community stability, I used methods from Keith et al. (2010). Bray-Curtis similarities were calculated for each tree, comparing the insect communities found on the particular tree in 2014 with the community on the same tree in 2015. Bray-Curtis similarities range from zero to one, where zero indicates that the two

communities are completely different from each other and one indicates that the two communities are identical.

To determine which aspects of insect communities were heritable traits of aspen genets, and to assess the heritability of various tree traits, I derived heritability calculations from the following mixed linear model (adapted from Robinson et al. 2012) with univariate community metrics:

$$x_{jkl} = u + b_j + g_k + e_{jkl}$$

where $x_{jkl}$ is the community metric (*e.g.,* Shannon index, all metrics measured per minute of survey time) for the *lth* individual tree from the *jth* experimental block and the *kth* genet. All effects are random, the grand mean is $u$, and $e_{jkl}$ is the error. The community metrics were transformed to fit normality assumptions using the boxCox function in the car package in R (Fox and Weisberg 2011, R Core Team 2017). I built the models in the lme4 package in R (Bates et al. 2015) and extracted the variance components. I then calculated the broad-sense heritability by dividing the genotypic variance by the sum of the model variances (genotypic, block, error). As evident within this calculation, measurement error influences phenotypic variation and can bias heritability estimates (*i.e.,* as error increases, heritability will decrease). Plant and insect metrics in this study were likely measured with different levels of precision, which may have affected subsequent heritability estimates. To derive 95% confidence intervals for heritability estimates, I bootstrapped these heritability statistics using the bootMer function in lme4 (Bates et al. 2015).

To further assess associations among insect groups, I quantified genotype- and phenotype-based correlations among abundances of various functional groups (aphids,

ants, free feeders, gallers, rollers, and miners). I used Spearman's rank correlations in the Hmisc package in R (Harrell et al. 2016). Genotype-based correlations used genotype-averaged insect data, whereas phenotype-based correlations used individual tree data.

To identify tree traits that were associated with insect community composition, I used both univariate and multivariate community methods. To first reduce statistical noise, I averaged the community and trait data by tree genet. For the univariate approach, I regressed BoxCox transformed (car package in R, Fox and Weisberg 2011) community metrics (*i.e.,* Shannon index, species richness, total abundance) on standardized tree traits (mean = 0, sd = 1). To select the particular traits that best explained variation in the community metrics, I used subset model selection with Bayesian information criterion (leaps package in R, Lumley 2017).

For the multivariate approach, I implemented multilevel models, which can assess how multiple tree traits structure insect communities as a whole as well as influence individual species (and/or insect groups), all in one model (Jackson et al. 2012). I analyzed both common insect species and functional groups (aphids, ants, free feeders, gallers, rollers, mines) in both years (2014 and 2015) with four separate multilevel models using glmer in the lme4 package in R (Bates et al. 2015). To better meet model assumptions (*e.g.,* linearity), I converted the insect data into presence/absence and used binomial multilevel models. I included standardized tree traits (mean = 0, sd = 1) as both fixed and random effects, and to detect potential non-linear relationships I also included quadratic trait variables. In these multilevel models, the fixed effect traits are variables that structure the entire community, while the random

effect traits influence the various insect species/groups differently. To fit the model, I used forward selection via the LMERConvenienceFunctions package in R (Tremblay and Ransijn 2015). A more detailed explanation of these multilevel model methods is provided in the supplemental material (Supplemental Fig. 1).

## Results

*Heritable and variable aspects of insect communities*

Insect community metrics varied widely across aspen genets at WisAsp (data averaged across 2014-5, Fig. 1). Shannon index, which accounts for species richness and evenness, varied 3.4-fold across aspen genets, while species richness varied 4.4-fold. Total insect abundance varied 8.5-fold across aspen genets, while community stability varied 4.3-fold. On average, insect community metrics (*i.e.,* Shannon index, richness, and abundance) were 31-56% higher in 2015 than in 2014.

Herbivorous insect and ant communities had low heritability ($H^2$ = 0.00-0.14, Table 2) on the aspen genets at WisAsp in 2014-5. Of the univariate community metrics, species richness was the most heritable while community stability exhibited no heritability. In addition, heritability estimates varied across insect functional groups (Table 3). Galling insects were the most heritable group, while leaf miners and free feeders showed little to no heritability across aspen genets. Heritability estimates of both community metrics (diversity and richness) and abundances of different functional groups were fairly consistent across years (varied by 1-6% across years, Tables 2 and 3).

*Correlations among insects: role of host plant genetics*

Of 60 possible pairwise comparisons among insect functional groups in 2014-15, only 10 exhibited strong correlations in abundance after correcting for multiple testing (Table 3). Of these significant correlations, most were phenotype-based species interactions, while significant genotype-based correlations were often stronger than their corresponding phenotype-based correlation. The strongest of these species interactions was between aphids and ants, followed by relatively large negative correlations between aphids and free feeding insects. Correlations between insect functional groups were fairly consistent across years, except for positive relationships among aphids and leaf-modifying insects, which were strong in one year and nearly non-existent in the other year.

*Tree traits shape insect communities, groups, and species*

Tree traits varied substantially across aspen genets for data averaged across 2014-15 (Fig. 2). Tree size varied 70-fold across genets. Individual leaf area varied 3.2-fold, while specific leaf area varied 1.7-fold. Timing of bud break and bud set varied across genets by 20 and 52 days, respectively. The average number of EFNs per leaf varied from zero to three across genets in 2014 (EFNs were only surveyed in 2014). Condensed tannin levels varied by 9.6-fold, while phenolic glycosides varied 13.2-fold. In contrast to defense chemistry, levels of foliar nitrogen (an index of protein) varied by only 1.6-fold.

Broad-sense heritabilities varied 2.9-fold across traits and were largest for timing of bud break and smallest for tree size (Table 2). Timing of bud set, individual and

specific leaf areas, density of EFNs, and foliar N all had moderate-sized heritabilities (0.32-0.56). Broad-sense heritabilities were high for defense phytochemicals (>0.53), and these estimates varied modestly (14-23%) across years.

Insect community metrics were shaped by multiple aspen traits at WisAsp in 2014-5 (Table 4). Metrics linked to species richness (Shannon index and total richness) were largely affected by tree size even though my survey methods were standardized by tree size (Fig. 3). Phytochemistry, bud phenology, and leaf area all contributed to shaping the community metrics, although traits that best explained these metrics varied considerably across years. In addition, the amount of community variation that could be explained by these models was noticeably higher for 2014 compared with 2015 models.

Several tree traits structured the presence/absence of common insect species and functional groups in aspen canopies in 2014-5 (Table 5). In order of decreasing relative importance: tree size, extra-floral nectary density, bud phenology, condensed tannins, and specific leaf area all significantly shaped insect species occurrences, while only tree size, condensed tannins, and foliar bud phenology shaped occurrences of insect functional groups. Levels of phenolic glycosides did not appear to influence the presence/absence of common insect species or functional groups in 2014-5. In addition, the tree traits that shaped aspen insect communities were fairly consistent across years. Most traits appeared to influence the occurrence of insect species/groups similarly, because few traits were selected as random effects (which allow for different coefficients for each insect species/group, whereas fixed effects supply one coefficient for all insect species/groups).

Insect functional groups responded to tree traits more similarly than did individual species (*i.e.,* more random effects for tree traits were included in the common species models compared with the functional-group models, Table 6). Of the few tree traits that appeared to affect the occurrence of insect species/groups differentially, foliar bud phenology was most prominent. In addition, particular leaf-modifying insects typically responded the most strongly to bud phenology traits and specific leaf area. Consistent with species richness models, all insect species responded positively with increased tree size in 2014 and 2015 and thus were more often present on larger trees.

**Discussion**

Previous work has shown that insect communities are shaped by a plant's phenotype and genotype (*e.g.,* Wimp et al. 2007, Barbour et al. 2015, Glassmire et al. 2016, Evans et al. 2016). My work expands on those findings by partitioning insect community structure to identify the most heritable components, comparing genotype- and phenotype-based species correlations, and quantifying the relative importance of a diverse set of plant traits in shaping insect communities, groups, and species. My findings show that insect communities overall had low and variable heritability estimates. Few genotype- and phenotype-based species interactions were identified. Of these significant relationships, both genotype- and phenotype-based species interactions were relatively strong between foundation insect species (aphids and ants) and other community members (*e.g.,* free feeders). Several tree traits influenced insect community metrics and the presence/absence of common insect species and functional groups. Most notably, tree size had a dramatic effect, with denser and more diverse

insect communities found on larger trees, while foliar bud phenology was the most important trait for differentially affecting insect species and functional groups.

*Heritable and variable aspects of insect communities*

Broad-sense heritability estimates ($H^2$) for community phenotypes vary widely in community genetics research, from 0.00 for the diversity and composition of endophytes on *Populus angustifolia* (Lamit et al. 2014) to 0.81 for the abundance of galling insects on *Populus tremula* (Bernhardsson et al. 2013). From eight different community genetic studies with a collective total of 95 community heritability estimates, 41 estimates were below 0.10 $H^2$ and on average community heritability was estimated at 0.23 $H^2$ (+/- 0.23 SD, Johnson and Agrawal 2005, Shuster et al. 2006, Whitham et al. 2008, Keith et al. 2010, Robinson et al. 2012, Dewoody et al. 2013, Bernhardsson et al. 2013, Lamit et al. 2014). Community metrics with the highest heritability estimates were typically tied to species richness and community dissimilarity between plants (*i.e.,* nonmetric multidimensional scaling axes from Bray-Curtis community dissimilarities, Shuster et al. 2006, Whitham et al. 2008, Bernhardsson et al. 2013). Moreover, community heritability estimates can vary considerably across environments and with time of sampling (Johnson and Agrawal 2005, Dewoody et al. 2013). In my study, insect community metrics had low heritability estimates overall, while a few aspects of the community, including richness, abundance of common insects, and abundance of galling insects were slightly heritable ($H^2$ = 0.10 – 0.15).

Across insect functional groups, heritability appeared to be largely influenced by the insect's mobility and relationship with the host plant. For instance, immobile leaf

gallers are intimately associated with their host tree (*i.e.,* live inside leaves, alter leaf development) and exhibited the highest heritability, while free feeders (not including aphids) are more mobile, less closely associated with their host tree and exhibited the lowest heritability. This pattern is similar to the findings of Bernhardsson et al. (2013) with herbivorous insect communities on European aspen.

After galling insects, aphids had the next highest estimated heritability. Aphid population dynamics and potential feedback loops with the host tree may underlie this heritability (*i.e.,* due to their rapid population growth and ability to disperse, aphids can readily adapt and respond to their host plant). In addition, aphids appear to be able to distinguish intraspecific differences in their host; different genotypes of aphids select different genotypes of their host plant, resulting in genotype-by-genotype interactions (Zytynska and Preziosi 2011).

*Correlations among insects: role of host plant genetics*

Relationships among insects were shaped by a few genotype- and phenotype-based species interactions. Of these, phenotype-based interactions were more abundant, while genotype-based interactions were stronger between particular insects (aphids, ants, free feeders) than the analogous phenotype-based relationships. Interestingly, the most heritable insect group, gallers, exhibited no relationships with other insect groups. This finding may indicate that gallers directly interact with the host plant but not with other insects on the tree. Mining species also showed little to no relationship with other insect groups. Potentially these species interact more within their functional group than across functional groups (*e.g.,* competitive interactions among

mining species for available foliage). Aphids and ants played a dominant role in structuring insect communities on aspen, consistent with the findings of (Wimp and Whitham 2012). For instance, aphids and ants had a negative genotype-based relationship with free-feeding herbivores, potentially via ant predation. I recognize that the nature of the work I conducted allowed for identification of only *associations*, not necessarily *interactions*, among insects groups. Previous work, however, has demonstrated such direct interactions in similar *Populus*-insect systems (Wimp and Whitham 2012).

These results are consistent with predictions of the "interacting foundation species hypothesis" (Keith et al. 2010, Lamit et al. 2015), which posits that interactions between key species (*i.e.,* aspen, aphids, and ants) can shape larger communities (insect herbivores). This hypothesis helps to distill complex community interactions to their core components and forms a basis for understanding community evolution (Whitham et al. 2006). My data show that even organisms with negligible heritability estimates (*e.g.,* free feeding insect herbivores) can still be structured by genotype-based plant effects through interactions with other foundation species (aphids and ants). Communities are predicted to evolve when genotype-based species interactions within the community change (Shuster et al. 2006). As I have shown with insect communities on aspen, and others (Keith et al. 2010, Lamit et al. 2015) have shown with various communities (*e.g.,* lichen, pathogen) on poplar, genotype-based species and community interactions can be relatively strong and therefore the potential for community evolution is likely.

The strength of plant genotype-based species interactions appears to be influenced by the type of community that is investigated. For instance, Maddox and Root (1987), Bernhardsson et al. (2013), and Barbour et al. (2015) surveyed *herbivorous* insect communities and found little evidence for significant genotype-based species interactions. However, Johnson and Agrawal (2005) investigated a more diverse community, including arthropod herbivores, omnivores, and predators, and found many strong genotype-based correlations among arthropod species. Thus, limiting the community to one trophic level may oversimplify and thus miss important foundation species (*e.g.,* pathogens in Busby et al. 2014, aphids and ants in my study) that govern community structure.

*Tree traits shape insect communities, groups, and species*

Four traits were especially prominent in shaping associated insect communities: tree size, foliar bud phenology, extra-floral nectaries and condensed tannins. Of these traits, tree size had the largest effect on community metrics (*e.g.,* richness) and presence/absence of insect species and functional groups. Larger trees had *denser* and more *diverse* insect communities than smaller trees, a finding that is consistent with willow and poplar studies (Barbour et al. 2015, Evans et al. 2016). Previous studies did not standardize insect surveys across plants of different sizes, and thus insect communities could appear more diverse and abundant on larger plants simply due to larger sampling areas and effort.

The positive relationship between tree size and insect species richness and abundance could be maintained through several potential mechanisms. For instance,

larger trees may have more available niches for insects to occupy (Campos et al. 2006). Also, in accordance with the Plant Vigor Hypothesis, insects may preferentially attack larger trees that are more vigorous than smaller trees (Price 1991, Rubert-Nason et al. 2017). In addition, larger trees may have more diverse communities than smaller trees because they provide a larger suitable habitat with a shorter distance to neighboring trees, in keeping with the theory of Island Biogeography (MacArthur and Wilson 1967).

Because tree size has been found to be the most important trait in shaping insect communities in this and similar studies, the heritability of tree size may set the upper bound for the heritability of associated insect communities (Barbour et al. 2015). If true, this could explain why insect community metrics had low heritability estimates in my study; tree size is one of the least heritable tree traits.

Foliar bud phenology was the most important trait in differentially affecting insect groups and species, especially leaf-modifying insects. Foliar phenology has also been shown to influence arthropod community structure and diversity in other *Populus* species (Robinson et al. 2012, Evans et al. 2016). Unlike tree size, foliar phenology is highly heritable and thus this trait is a promising candidate for “genes to ecosystems” research (Wymore et al. 2011). Several quantitative trait loci (QTL) have been identified in *Populus* that are tied to bud set and break, including the *FLOWERING LOCUS T2* (FT2; Rohde et al. 2011, McKown et al. 2014, Wang et al. 2017). Knockout and/or genetic modification studies could shed light on the community- and ecosystem-level effects of these phenology-regulating genes. In addition, insect sensitivity to foliar phenology will likely have important consequences with climate change and advancing bud break (*e.g.,* Evans et al. 2016, Falk 2017).

The density of extra-floral nectaries (EFNs) had a large negative effect on the occurrence of insect species and functional groups. This may be due to increased parasitism, since EFNs attract parasitoids. EFNs are also thought to attract ants, although in my study, ant populations responded more to the presence of aphids than to EFNs.

High levels of condensed tannins were related to decreased insect richness, total abundance, and incidence of species and functional groups. Insect herbivores may have avoided or responded with decreased fitness (growth, survival, reproduction) on high-tannin trees. While previous research on aspen-insect interactions has not found strong evidence that tannins negatively influence insect herbivores (Lindroth and St. Clair 2013), those studies typically examined effects on free feeding Lepidoptera, which are not prominent members of the insect communities found in my study. Negative effects of condensed tannins have been shown, however, for chrysomelid beetles and aphids (Donaldson and Lindroth 2007, Vigue and Lindroth 2010, Rubert-Nason et al. 2017), including *Chaitophorus stevensis*, a common species in my data. The negative effects of condensed tannins on aspen insect communities may also derive from a trade-off between tree growth and investment in chemical defense, especially condensed tannin concentrations (Cole et al. 2016, Barker et al. unpublished data) as tannin-rich trees tended to have smaller sizes.

In my study, levels of phenolic glycosides (salicinoids), the signature defense compounds in the Salicaceae, did not play a strong role in structuring associated insect communities. These results are similar to those of Robinson et al. (2012), who found no relationship between arthropod abundance/richness and total phenolics (Folin-

Ciocalteau assay) in a common garden of *Populus tremula* genotypes. Volf et al. (2015) also found no relationship between salicinoid levels and insect abundance in a *Salix* common garden, although *Salix* species with higher levels of salicinoids supported less diverse insect communities, due to exclusion of generalists. The absence of relationship between insect community metrics and phenolic glycoside levels in my study may have stemmed from several factors. First, many of my common insect species were specialists of aspen and thus may not be negatively affected by the compounds (*e.g.,* Kleemann et al. 2011, Robinson et al. 2012; Volf et al. 2015). Second, a subset of specialist insects, the salicinoid-sequestering species that select for high levels of phenolic glycosides may influence community diversity and abundance, but these insects were rare in our study. Third, the effects of chemical defense compounds on insect herbivores may vary throughout the growing season. Insects are likely particularly sensitive to phytochemistry at critical life stages (e.g., neonate establishment) and my surveys may not have captured these critical moments (*e.g.,* Wimp et al. 2007). Levels of phenolic glycosides and condensed tannins have been shown to link to the abundance of sap sucking and leaf chewing insects at particular time points in the season (early, mid, and/or late; Brito 2017).

Results from my work could indicate that defense compounds may not be as important in structuring insect communities as originally thought. In community genetics studies that have surveyed the effects of a diverse array of plant traits on structuring associated insect communities, chemical defense was typically found to be less important compared with other traits (*e.g.,* growth, size, etc., Robinson et al. 2012, Barbour et al. 2015). Similarly, in a meta-analysis of the effects of genetically-variable

plant traits on herbivores, chemical defenses were not associated with herbivore susceptibility, while plant size and morphology traits were a strong predictor of herbivore susceptibility in woody plant species (Carmona et al. 2011). Chemical defense traits may function like a filter, determining which insects can associate with particular plant species, rather than affecting the abundance and diversity of those that do. While chemical defenses can structure some insect communities (*e.g.,* Wimp et al. 2007, Dewoody et al. 2013, Lexer et al. 2013, Volf et al. 2015, Glassmire et al. 2016), other plant traits, especially size and phenology traits, may have stronger effects in some systems.

I expect that the relationships between aspen and associated insect communities will change with tree ontogeny and stand maturity (*e.g*., Barton and Koricheva 2010). As aspen age, their phytochemistry shifts: levels of condensed tannins increase while phenolic glycosides decrease (Donaldson et al. 2006). Similarly, expression of extra floral nectaries declines with age (Wooley et al. 2007). Across the two years of my study, insect community metrics (Shannon index, richness, abundance) across aspen genets increased in the second year of sampling. This increase may have been in part due to differences in environment and insect population dynamics, but was most likely due to increases in tree size. I expect that increases in species richness and abundance will continue as the trees mature, but that with increasing size, communities will eventually saturate. At this point, other tree traits would likely become more important in structuring insect community composition. In addition, I found that community stability across years was relatively high over all aspen replicates (mean = 0.68). This result suggests that the composition of insect communities on individual trees was fairly

similar from year to year. However, community stability was not heritable across years, indicating that differences in stability across genets were primarily shaped by environmental factors. Genotype-based species interactions may shift as the stand matures, resulting in community evolution.

Increasingly, genotypic variation within plant species has been shown to shape associated communities, from simple metrics such as organism abundance to more complex interactions across co-associated communities (*e.g.,* lichen and microbes; Zhang et al. 2014, Lamit et al. 2015). My findings advance the understanding of community genetics by demonstrating that particular metrics of community structure are variously heritable, that communities are shaped by a few strong relationships among key species (aphids and ants), and that across a diverse set of plant traits, growth and phenology can be the most important factors shaping associated insect communities. Moving forward, community genetics research should dissect plant genotypic variation further to identify the genes that structure associated communities (Dewoody et al. 2013, Bernhardsson et al. 2013, Zinkgraf et al. 2016), explore the evolutionary drivers of these genetic mechanisms, and assess how global environmental change may select variants of these genes and shape their extended effects.

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**Table 1.** Common insect species found on aspen in the WisAsp common garden in 2014-5. Information (identification, life history, images) for these species can be found at https://aspeninsects.wordpress.com/

| Order | Family | Genus | Species | Feeding Guild |
|---|---|---|---|---|
| Hymenoptera | Formicidae | *Lasius* | *neoniger* | Aphid tender |
| | | | *alienus* | Aphid tender |
| | | *Formica* | *glacialis* | Aphid tender |
| | Tenthredinidae | *Nematus* | *Sp. 1* | Free feeder |
| | | *Phyllocolpa* | *Sp. 1* | Leaf miner |
| Diptera | Agromyziidae | *Paraphytomyza* | *populicola* | Leaf miner |
| | Cecidomyiidae | *Harmandia* | *Sp. 1* | Leaf galler |
| | | *Prodiplosis* | *morrisi* | Leaf roller |
| Hemiptera | Aphididae | *Chaitophorus* | *populicola* | Phloem feeder |
| | | | *stevensis* | Phloem feeder |
| Coleoptera | Megalopodidae | *Zeugophora* | *scutellaris* | Leaf miner |
| Lepidoptera | Gracilliaridae | *Phyllonorycter* | *tremuloidiella* | Leaf miner |
| | | *Caloptilia* | *stigmatella* | Leaf miner |
| | | *Phyllocnistis* | *populiella* | Leaf miner |
| | Lyonetiidae | *Paraleucoptera* | *albella* | Leaf miner |
| | Notodontidae | *Clostera* | *albosigma* | Leaf roller |
| | | *Gluphisia* | *septentrionis* | Free feeder |
| | Noctuidae | *Acronicta* | *lepusculina* | Free feeder |
| | Nepticulidae | *Ectoedemia* | *populella* | Petiole galler |
| | Tortricidae | *Choristoneura* | *rosaceana* | Leaf roller |

**Table 2.** Broad-sense heritability ($H^2$) estimates for insect community metrics (richness, abundance, Shannon index, community stability) and tree traits (tree size, bud break, bud set, extra-floral nectary density per leaf, specific leaf area, individual leaf area, and foliar levels of condensed tannins, phenolic glycosides, and nitrogen) for WisAsp genets (N = 328) in 2014 and 2015. Values in parentheses are the 95% bootstrapped confidence intervals of $H^2$.

| **Trait** | **Broad-sense heritability ($H^2$)** | |
|---|---|---|
| | 2014 | 2015 |
| Richness | 0.14 (0.07-0.20) | 0.11 (0.04-0.17) |
| Abundance | 0.10 (0.03-0.16) | 0.11 (0.04-0.17) |
| Shannon index | 0.09 (0.02-0.15) | 0.09 (0.02-0.15) |
| Community stability | 0.04 (-0.02-0.10) | |
| Tree size ($cm^3$) | 0.30 (0.22-0.38) | 0.39 (0.31-0.47) |
| Julian bud break date | 0.84 (0.81-0.87) | 0.86 (0.84-0.89) |
| Julian bud set date | 0.44 (0.37-0.52) | 0.48 (0.41-0.55) |
| EFN density per leaf | 0.45 (0.39-0.53) | NA |
| Specific leaf area ($cm^2g^{-1}$) | 0.41 (0.33-0.49) | 0.43 (0.33-0.54) |
| Individual leaf area ($cm^2$) | 0.44 (0.36-0.52) | 0.56 (0.49-0.63) |
| Condensed tannins (%dw) | 0.67 (0.60-0.75) | 0.53 (0.46-0.60) |
| Phenolic glycosides (%dw) | 0.82 (0.78-0.85) | 0.59 (0.53-0.66) |
| Nitrogen (%dw) | 0.47 (0.40-0.55) | 0.32 (0.20-0.43) |

**Table 3.** Phenotype-based and genotype-based (italicized) Spearman rank correlations among groups of insects on aspen (328 genets) in the WisAsp common garden in 2014 (top) and 2015 (bottom). Bolded correlations are statistically significant after a false discovery rate (FDR) correction (P < 0.05). Broad-sense heritabilities (with 95% confidence intervals) for the abundances of the insect groups across aspen genets are given in the diagonal (gray background).

| 2014 | Aphid | Ant | Free feeder | Galler | Roller | Miner |
|---|---|---|---|---|---|---|
| Aphid | 0.08 (0.02 – 0.15) | ***0.54*** | ***-0.35*** | *0.03* | *0.13* | *0.06* |
| Ant | **0.48** | 0.04 (-0.02 – 0.09) | *-0.11* | *-0.01* | *0.13* | *0.00* |
| Free feeder | **-0.32** | **-0.14** | 0.05 (0.03 – 0.16) | *0.05* | *-0.06* | *-0.05* |
| Galler | 0.00 | 0.01 | 0.02 | 0.15 (0.08 – 0.22) | *0.00* | *-0.04* |
| Roller | **0.13** | 0.04 | -0.05 | 0.02 | 0.04 (-0.02 – 0.10) | *0.03* |
| Miner | **0.12** | -0.01 | 0.04 | 0.01 | 0.03 | 0.05 (-0.01 – 0.10) |

| 2015 | Aphid | Ant | Free feeder | Galler | Roller | Miner |
|---|---|---|---|---|---|---|
| Aphid | 0.09 (0.02 – 0.15) | ***0.61*** | ***-0.25*** | *0.09* | *0.06* | *-0.02* |
| Ant | **0.53** | 0.08 (0.02 – 0.15) | *-0.17* | *0.06* | *0.11* | *-0.03* |
| Free feeder | **-0.12** | -0.02 | 0.02 (0.03 – 0.16) | *0.01* | *-0.03* | *0.00* |
| Galler | 0.05 | 0.00 | -0.02 | 0.13 (0.07 – 0.20) | *-0.06* | *0.01* |
| Roller | 0.03 | 0.04 | 0.05 | 0.04 | 0.10 (0.02 – 0.14) | *-0.01* |
| Miner | 0.01 | -0.03 | 0.01 | 0.04 | **0.13** | 0.03 (-0.02 – 0.07) |

**Table 4.** Key aspen traits that shaped univariate insect community metrics (*i.e.,* Shannon index, species richness, total abundance) at WisAsp in 2014 and 2015. Tree traits were standardized (mean 0 and SD 1) and community metrics were standardized by survey time. Models were selected using subset model selection and Bayesian information criteria (BIC).

| Year | Community metric | Traits | Coef | Std. error | t | P | Model adjusted R2 |
|---|---|---|---|---|---|---|---|
| 2014 | Shannon index | Average size | 0.197 | 0.027 | 7.372 | 0.000 | 0.203 |
| | | Specific leaf area | 0.082 | 0.029 | 2.835 | 0.005 | |
| | | Total phenolic glycosides | 0.045 | 0.025 | 1.802 | 0.073 | |
| | Richness | Average size | 0.211 | 0.023 | 9.156 | 0.000 | 0.212 |
| | Abundance | Average size | 0.002 | 0.001 | 3.110 | 0.002 | 0.044 |
| | | Bud break date | -0.001 | 0.001 | -1.727 | 0.085 | |
| 2015 | Shannon index | Average size | 0.047 | 0.011 | 4.362 | 0.000 | 0.095 |
| | | Bud set date | 0.031 | 0.011 | 2.779 | 0.006 | |
| | Richness | Average size | 0.211 | 0.048 | 4.386 | 0.000 | 0.137 |
| | | Bud set date | 0.127 | 0.048 | 2.623 | 0.009 | |
| | | Total defense chemistry | -0.149 | 0.057 | -2.588 | 0.010 | |
| | Abundance | Total defense chemistry | -0.144 | 0.055 | -2.622 | 0.009 | 0.039 |
| | | Individual leaf area | 0.101 | 0.042 | 2.413 | 0.016 | |

Total phenolic glycosides = combined levels of salicortin and tremulacin

Total defense chemistry = combined levels of condensed tannings, salicortin, and tremulacin

**Table 5.** Fixed and random effect coefficients (see footnote below) for the best-fit binomial multilevel models for presence/absence of common insect species (left) or functional groups (right) in 2014 and 2015. Quadratic coefficients are shown with superscripts ($^2$). Bolded coefficients are statistically significant ($P < 0.05$).

| Variable | Species-level | | | | Functional group-level | | | |
|---|---|---|---|---|---|---|---|---|
| | 2014 | | 2015 | | 2014 | | 2015 | |
| | Fixed | Random | Fixed | Random | Fixed | Random | Fixed | Random |
| Intercept | 0.062 | 1.463 | 0.499 | 2.705 | **1.494** | 1.418 | **2.696** | 2.159 |
| Size | **0.506** | … | **0.589** | 0.028 | **1.239** | … | **1.016** | … |
| Size$^2$ | **-0.171** | … | **-0.248** | … | **-0.334** | … | **-0.411** | … |
| EFN density | **-0.361** | 0.056 | … | … | … | … | … | … |
| EFN density$^2$ | **0.294** | … | … | … | … | … | … | … |
| SLA | 0.155 | … | -0.071 | 0.073 | … | … | … | … |
| SLA$^2$ | **-0.296** | … | … | … | … | … | … | … |
| Bud break date | -0.021 | … | -0.059 | 0.042 | 0.096 | … | -0.149 | … |
| Bud break date$^2$ | **0.081** | … | **0.121** | … | **0.188** | … | **0.284** | … |
| Bud set date | **0.217** | 0.039 | 0.091 | … | 0.164 | 0.201 | … | … |
| Bud set date$^2$ | … | … | **-0.221** | … | … | … | … | … |
| Condensed tannins | -0.036 | … | **-0.176** | … | … | … | 0.081 | … |
| Condensed tannins$^2$ | **-0.173** | … | **-0.192** | … | … | … | **-0.361** | … |
| Nitrogen | … | … | **-0.221** | … | … | … | … | … |

Fixed effects influence the occurrence of all insects in the community similarly, while random effects differentially influence the occurrence of insects species/groups

SLA = Specific leaf area

EFN = Extra-floral nectary

… = effect not included in the model

**Table 6.** Multilevel model random effect coefficients by common insect species (left) or functional group (right) in both 2014 and 2015. Coefficients for tree traits are the random effect plus the fixed effect estimate, which accounts for the mean slope. For each random effect tree trait, the insect that responds the most strongly has a bolded coefficient value.

| Species | 2014 | | | 2015 | | | | Functional group | 2014 | | 2015 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intercept | EFN density | Bud set date | Intercept | Size | SLA | Bud break date | | Intercept | Bud set date | Intercept |
| *Chaitophorus populicola* | 0.045 | -0.166 | 0.293 | -0.647 | 0.483 | 0.223 | -0.031 | Aphid | 0.069 | 0.546 | -0.632 |
| *Chaitophorus stevensis* | 0.934 | -0.559 | 0.278 | 1.118 | **0.721** | -0.183 | -0.079 | | | | |
| *Formica glacialis* | … | … | … | -2.078 | 0.510 | -0.301 | -0.015 | Ant | -2.195 | 0.312 | -1.727 |
| *Lasius neoniger* | -1.413 | -0.362 | 0.300 | -0.531 | 0.678 | 0.061 | -0.177 | | | | |
| *Lasius alienus* | … | … | … | -1.763 | 0.570 | -0.071 | -0.162 | | | | |
| *Acronicta lepusculina* | … | … | … | -1.770 | 0.503 | -0.202 | -0.195 | Free feeder | -0.071 | 0.032 | -1.319 |
| *Gluphisia septentrionis* | 0.881 | -0.511 | 0.250 | … | … | … | … | | | | |
| *Nematus sp. 1* | -2.013 | -0.137 | 0.116 | … | … | … | … | | | | |
| *Harmandia sp. 1* | -1.218 | -0.147 | 0.158 | -0.576 | 0.667 | 0.114 | 0.004 | Galler | -0.340 | -0.402 | -0.094 |
| *Ectoedemia populella* | 0.963 | -0.274 | -0.022 | 1.575 | 0.602 | -0.310 | -0.177 | | | | |
| *Phyllocolpa sp. 1* | 1.627 | -0.293 | 0.237 | 2.557 | 0.593 | 0.171 | -0.057 | Roller | 1.035 | **0.566** | 1.894 |
| *Clostera albosigma* | -0.186 | -0.358 | 0.291 | -2.019 | 0.545 | 0.051 | **0.209** | | | | |
| *Choristoneura rosaceana* | -1.201 | -0.544 | 0.114 | 0.224 | 0.423 | -0.046 | -0.183 | | | | |
| *Phyllocnistis populiella* | -1.005 | **-0.608** | **0.476** | 1.273 | 0.635 | 0.086 | -0.029 | Miner | 1.292 | -0.101 | 1.518 |
| *Caloptilia stigmatella* | 0.418 | -0.292 | 0.223 | 0.627 | 0.622 | -0.271 | 0.148 | | | | |
| *Paraleucoptera albella* | … | … | … | -2.247 | 0.545 | -0.206 | -0.098 | | | | |
| *Phyllonorycter tremuloidiella* | 1.915 | -0.458 | 0.233 | 2.156 | 0.644 | -0.404 | -0.156 | | | | |
| *Paraphytomyza populiola* | 0.239 | -0.329 | 0.078 | 2.357 | 0.647 | -0.109 | -0.011 | | | | |
| *Zeugophora scutellaris* | … | … | … | -1.270 | 0.516 | **-0.500** | -0.184 | | | | |
| *Prodiplosis morrisi* | … | … | … | 0.875 | 0.672 | 0.153 | 0.159 | | | | |

*Caloptilia stigmatella* creates both leaf mines and rolls; here we characterize them as a leaf-mining species only

SLA = Specific leaf area

EFN = Extra-floral nectary

… = effect not included in the model

**Figure 1.** Variation in insect community metrics (species richness, Shannon Index, total abundance, and community stability) across aspen genets at WisAsp in 2014 and 2015. Each black point is the average value across years and tree replicates, and the gray shading represents the standard deviation. All plots are ordered from low to high genet values.

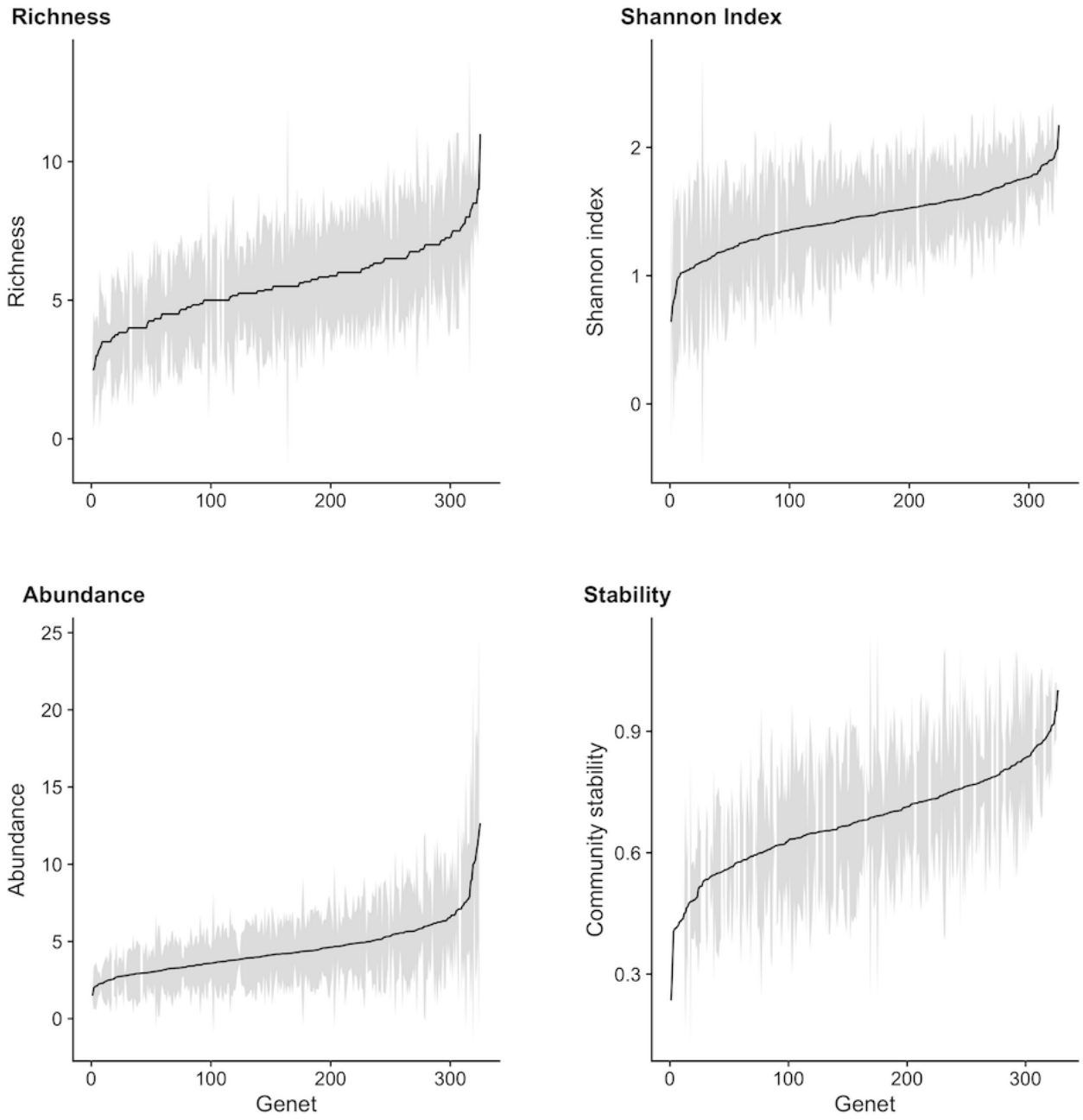

Richness
Richness
0
5
10
0
100
200
300
Shannon Index
Shannon index
0
1
2
0
100
200
300
Abundance
Abundance
0
5
10
15
20
25
0
100
200
300
Genet
Stability
Community stability
0.3
0.6
0.9
0
100
200
300
Genet

**Figure 2.** Variation in tree traits (tree size, Julian bud break date, Julian bud set date, density of extra-floral nectaries per leaf [EFN], specific leaf area [SLA], levels of foliar condensed tannins [CT], levels of foliar phenolic glycosides [PG, salicortin and tremulacin combined], levels of foliar nitrogen [N]) across aspen genets at WisAsp in 2014 and 2015. Each black point is the average value across years and tree replicates, and the gray shading represents the standard deviation. All plots are ordered from low to high genet values.

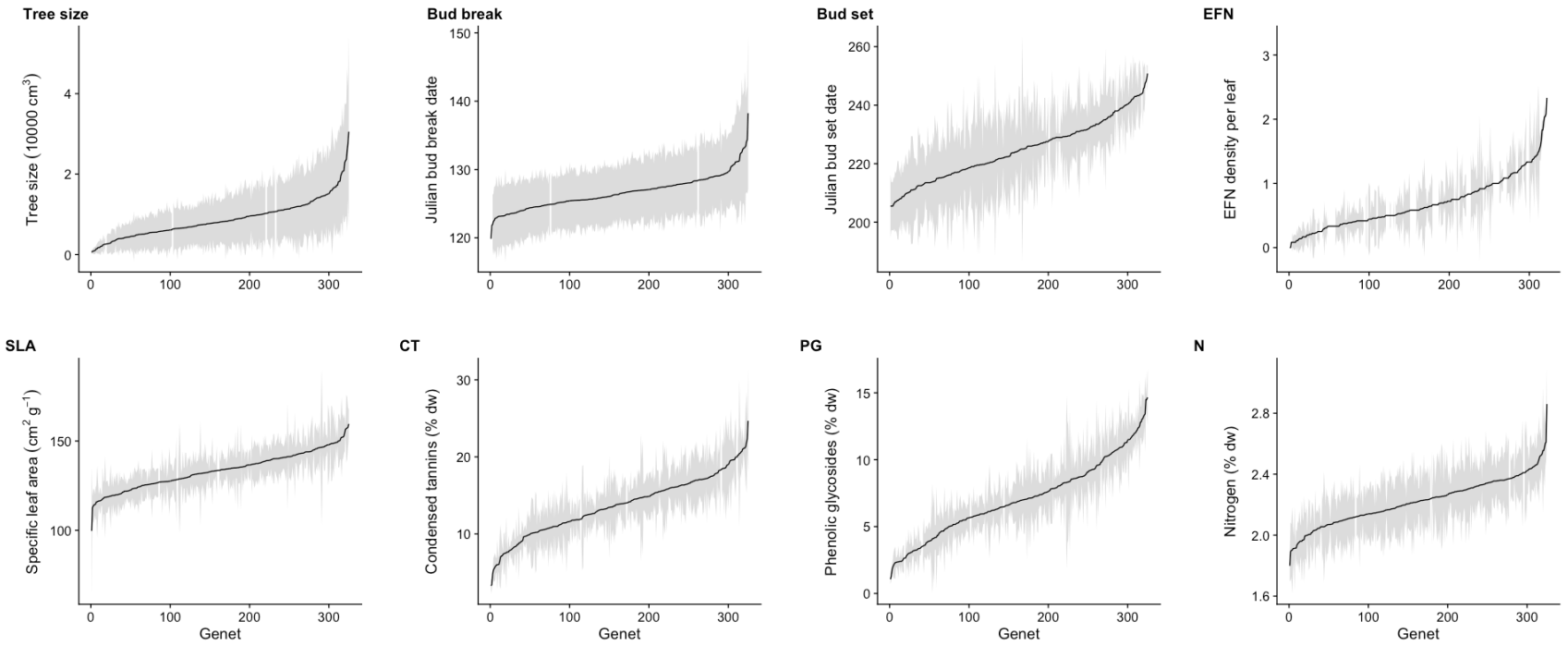
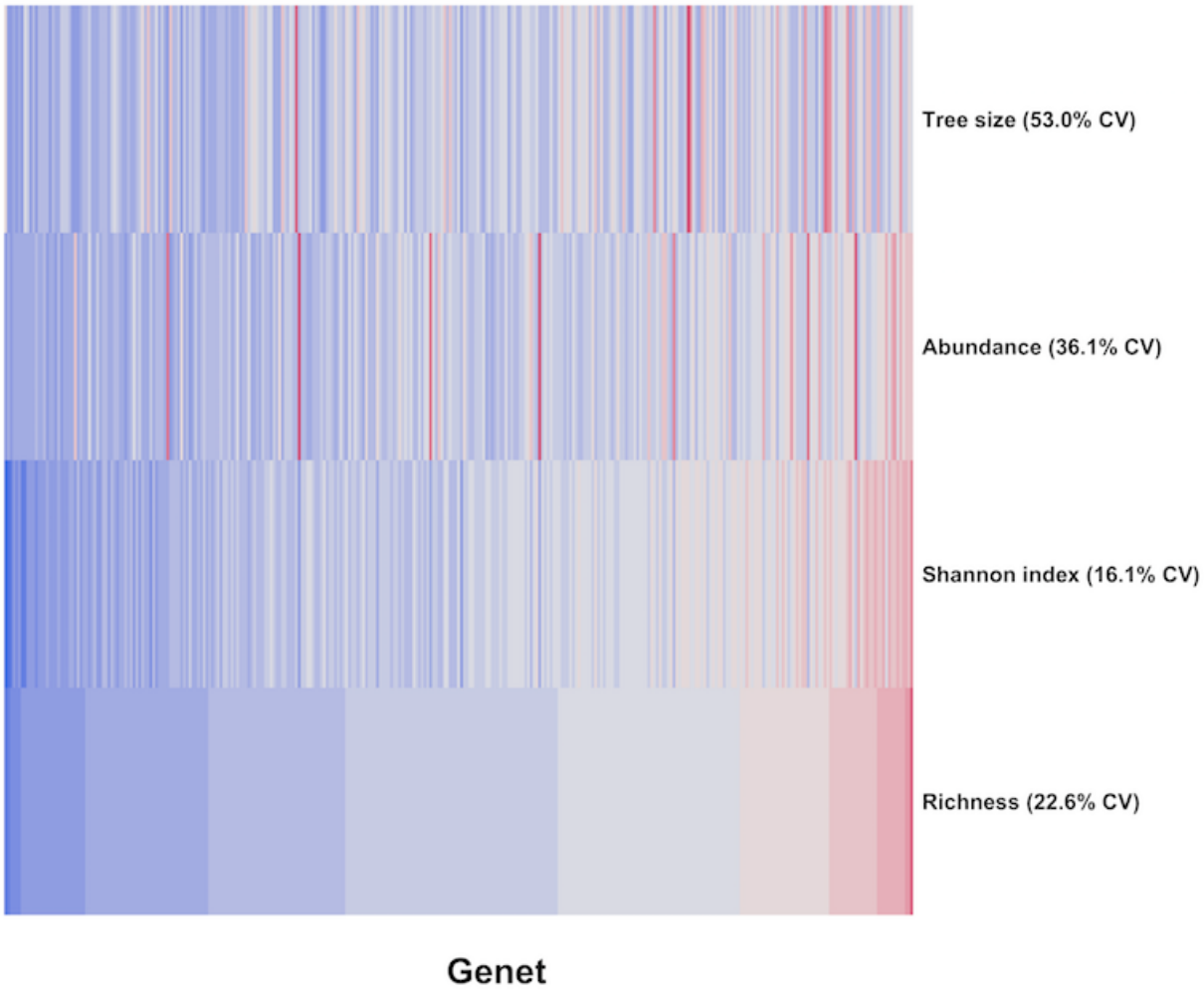

Tree size
Tree size (10000 cm3)
Bud break
Julian bud break date
Bud set
Julian bud set date
EFN
EFN density per leaf
SLA
Specific leaf area (cm2 g-1)
CT
Condensed tannins (% dw)
PG
Phenolic glycosides (% dw)
N
Nitrogen (% dw)
Genet

**Figure 3.** Heatmap of standardized insect community metrics (richness, Shannon index, and total abundance) and tree size (volume) across aspen genets at WisAsp in 2014 and 2015. Each standardized value is the mean across years and genet replicates. The genets are all ordered from low (blue) to high (red) insect species richness (values are binned into 12 different shades of color). Coefficients of variation (CV) are given after each metric.


Tree size (53.0% CV)
Abundance (36.1% CV)
Shannon index (16.1% CV)
Richness (22.6% CV)
Genet

**Supplemental Table 1.** Resources used to identify insect specimens from WisAsp.

| Insect group | Identification book / reference |
|---|---|
| **General insects** | Arnett, R.H. Jr. 2000. American insects: a handbook of the insects of North America and Mexico. 2nd ed., Boca Raton (FL): CRC Press LLC. |
| | Castner, J.L. 2000. Photographic atlas of entomology and guide to insect identification. Gainesville (FL): Feline Press. |
| | Chu, H.F. and Jaques, H.E. 1949. How to know the immature insects. Dubuque (IA) WM. C. Brown Company. |
| | Eiseman, C. and Charney, N. 2010. Tracks and signs of insects and other invertebrates: a guide to North American species. 1st ed., Mechanicsburg (PA): Stackpole Books. |
| | Felt. E.P. 1917. Key to American insect galls. Albany (NY): University of the State of New York Press. |
| | Hahn, J. 2009. Insects of the north woods. 1st ed., Duluth (MN): Kollath and Stensaas Publishing. |
| | Rose, A.H. and Lindquist, O.H. 1997. Insects of eastern hardwood trees. Ottawa: Natural Resources Canada. |
| **Hymenoptera** | Coovert, G.A. 2005. The ants of Ohio (Hymenoptera: Formicidiae). Ohio Biological Survey Bulletin New Series 15(2). |
| | Fisher, B.L. and Cover, S.P. 2007. Ants of North America: a guide to the genera. Berkeley and Los Angeles (CA): University of California Press. |
| **Diptera** | Gagné, R.J. 1989. The plant-feeding gall midges of North America. Ithaca (NY): Cornell University Press. |
| **Coleoptera** | Evans, A.V. 2014. Beetles of eastern North America. Princeton (NJ): Princeton University Press. |
| **Lepidoptera** | Beadle, D. and Leckie, S. 2012. Peterson field guide to moths of northeastern North America. New York (NY): Houghton Mifflin Harcourt Publishing Company. |
| | Sogaard, J. 2009. Moths and caterpillars of the north woods. 1st ed., Duluth (MN): Kollath and Stensaas Publishing. |
| | Wagner, D.L. 2005. Caterpillars of eastern North America. Princeton (NJ): Princeton University Press. |
| | Wagner, D.L., Schweitzer, D.F., Sullivan, J.B., and Reardon, R.C. 2011. Owlet caterpillars of eastern North America. 1st ed., Princeton (NJ): Princeton University Press. |

**Supplemental Table 2.** Complete list of insect species and morpho-species found on aspen at the WisAsp common garden in 2014-5. Abundance values are the total number of insects counted for the entire WisAsp garden.

| Order | Family | Genus | Species | Functional group | 2014 Abundance | 2015 Abundance |
|---|---|---|---|---|---|---|
| Coleoptera | Buprestidae | *Poecilonota* | *cyanipes* | Wood-modifying | 6 | 3 |
| | | Buprestidae | sp. 1 | Wood-modifying | 0 | 1 |
| | Cerambicidae | *Brachysomida* | *bivittata* | Wood-modifying | 0 | 1 |
| | | *Saperda* | *inornata* | Wood-modifying | 0 | 8 |
| | Chrysomelidae | *Leptinotarsa* | *decemlineata* | Free-feeding | 38 | 15 |
| | | *Plagiodera* | *versicolora* | Free-feeding | 0 | 2 |
| | | *Chrysomela* | *crotchi* | Free-feeding | 4 | 10 |
| | | *Rhabdopterus sp.* | | Free-feeding | 0 | 0 |
| | | *Chaetocnema sp.* | | Free-feeding | 0 | 3 |
| | | *Crepidodera* | *nana* | Free-feeding | 11 | 16 |
| | | *Diabrotica* | *barberi* | Free-feeding | 4 | 2 |
| | | *Diabrotica* | *undecimpunctata* | Free-feeding | 3 | 0 |
| | | *Diabrotica* | *virgifera* | Free-feeding | 7 | 0 |
| | | Galerucinae | sp. 1 | Free-feeding | 0 | 2 |
| | | Chrysomelidae | sp. 1 | Free-feeding | 0 | 0 |
| | | Chrysomelidae | sp. 2 | Free-feeding | 1 | 1 |
| | | Chrysomelidae | sp. 3 | Free-feeding | 0 | 4 |
| | | Chrysomelidae | sp. 4 | Free-feeding | 7 | 8 |
| | | Chrysomelidae | sp. 5 | Free-feeding | 2 | 23 |
| | | Chrysomelidae | sp. 6 | Free-feeding | 1 | 0 |
| | Curculionidae | *Polydrusus sp.* | | Free-feeding | 0 | 1 |
| | | *Tachyerges* | *salicis* | Leaf-modifying | 32 | 27 |
| | | Curculionidae | sp. 1 | Free-feeding | 4 | 1 |
| | | Curculionidae | sp. 2 | Free-feeding | 1 | 0 |
| | Elateridae | *Limonius sp.* | | Free-feeding | 0 | 2 |
| | Megalopodidae | *Zeugophora* | *scutellaris* | Leaf-modifying | 18 | 196 |
| | Scarabaeidae | *Popillia* | *japonica* | Free-feeding | 30 | 29 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Diptera | Agromyzidae | *Paraphytomyza* | *populicola* | Leaf-modifying | 503 | 1867 |
| | Cecidomyiidae | *Harmandia sp.* | | Leaf-modifying | 667 | 1123 |
| | | *Prodiplosis* | *morrisi* | Leaf-modifying | 16 | 1240 |
| Hemiptera | Aphididae | *Aphis* | *maculatae* | Free-feeding | 0 | 1228 |
| | | *Chaitophorus* | *populicola* | Free-feeding | 21204 | 28088 |
| | | *Chaitophorus* | *stevensis* | Free-feeding | 16205 | 26713 |
| | | Aphididae | sp. 1 | Free-feeding | 30 | 0 |
| | | Aphididae | sp. 2 | Free-feeding | 11 | 0 |
| | Cicadellidae | *Graphocephala* | *coccinea* | Free-feeding | 1 | 0 |
| | | Cicadellidae | sp. 1 | Free-feeding | 1 | 1 |
| | | Cicadellidae | sp. 2 | Free-feeding | 5 | 5 |
| | | Cicadellidae | sp. 3 | Free-feeding | 3 | 1 |
| | | Cicadellidae | sp. 4 | Free-feeding | 10 | 1 |
| | | Cicadellidae | sp. 5 | Free-feeding | 7 | 7 |
| | | Cicadellidae | sp. 6 | Free-feeding | 1 | 0 |
| | | Cicadellidae | sp. 7 | Free-feeding | 1 | 1 |
| | | Cicadellidae | sp. 8 | Free-feeding | 1 | 0 |
| | | Cicadellidae | sp. 9 | Free-feeding | 1 | 0 |
| | | Cicadellidae | sp. 10 | Free-feeding | 0 | 1 |
| | | Cicadellidae | sp. 11 | Free-feeding | 0 | 3 |
| | | Cicadellidae | sp. 12 | Free-feeding | 0 | 1 |
| | | Cicadellidae | sp. 13 | Free-feeding | 0 | 1 |
| | | Cicadellidae | sp. 14 | Free-feeding | 0 | 1 |
| | | Cicadellidae | immatures | Free-feeding | 5 | 5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Cicadidae | *Tibicen* | *canicularis* | Free-feeding | 1 | 1 |
| | Cixiidae | Cixiidae | sp. 1 | Free-feeding | 2 | 0 |
| | | Cixiidae | sp. 2 | Free-feeding | 0 | 10 |
| | | Cixiidae | immatures | Free-feeding | 0 | 1 |
| | Delphacidae | Delphacidae | sp. 1 | Free-feeding | 4 | 11 |
| | | Delphacidae | sp. 2 | Free-feeding | 3 | 12 |
| | | Delphacidae | sp. 3 | Free-feeding | 2 | 0 |
| | | Delphacidae | sp. 4 | Free-feeding | 2 | 0 |
| | | Delphacidae | sp. 5 | Free-feeding | 1 | 1 |
| | | Delphacidae | sp. 6 | Free-feeding | 0 | 17 |
| | | Delphacidae | sp. 7 | Free-feeding | 0 | 2 |
| | | Delphacidae | sp. 8 | Free-feeding | 0 | 3 |
| | | Delphacidae | sp. 9 | Free-feeding | 0 | 3 |
| | | Delphacidae | sp. 10 | Free-feeding | 0 | 1 |
| | | Delphacidae | sp. 11 | Free-feeding | 0 | 0 |
| | Membracidae | *Enchenopa* | *binotata* | Free-feeding | 31 | 11 |
| | | *Telamona* | *tremulata* | Free-feeding | 3 | 9 |
| | | *Ceresa* | *alta* | Free-feeding | 32 | 6 |
| | Miridae | *Lygus* | *lineolaris* | Free-feeding | 2 | 0 |
| | | Miridae | sp. 1 | Free-feeding | 1 | 1 |
| | | Miridae | sp. 2 | Free-feeding | 1 | 3 |
| | | Miridae | sp. 3 | Free-feeding | 1 | 0 |
| | | Miridae | sp. 4 | Free-feeding | 2 | 0 |
| | | Miridae | nymphs | Free-feeding | 1 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Pentatomidae | *Brochymena* | *arborea* | Free-feeding | 4 | 18 |
| | | *Chinavia* | *hilaris* | Free-feeding | 3 | 3 |
| | | *Euschistus* | *quadrator* | Free-feeding | 4 | 3 |
| | | *Euschistus* | *tristigmus* | Free-feeding | 1 | 1 |
| | Stink bug eggs | | | | 485 | 416 |
| | Stink bug nymphs | | | | 10 | 45 |
| | Pseudococcidae | Pseudococcidae | sp. 1 | Free-feeding | 0 | 1 |
| Hymenoptera | Cimbicidae | Cimbicidae | sp. 1 | Free-feeding | 0 | 2 |
| | Formicidae | *Camponotus* | *noveboracensis* | Ant | 11 | 56 |
| | | *Formica* | *glacialis* | Ant | 51 | 657 |
| | | *Formica* | *montana* | Ant | 226 | 217 |
| | | *Lasius* | *alienus* | Ant | 231 | 542 |
| | | *Lasius* | *neoniger* | Ant | 484 | 1237 |
| | | *Prenolepis* | *imparis* | Ant | 256 | 131 |
| | Myrmicinae | Myrmicinae | sp. 1 | Ant | 0 | 0 |
| | | Myrmicinae | sp. 2 | Ant | 0 | 7 |
| | Tenthredinidae | *Caliroa sp.* | sp. 1 | Free-feeding | 0 | 4 |
| | | *Nematus* | *hudsoniimagnus* | Free-feeding | 1 | 19 |
| | | *Nematus* | *limbatus* | Free-feeding | 0 | 37 |
| | | *Nematus* | sp. 1 | Free-feeding | 70 | 38 |
| | | *Phyllocolpa* | sp. 1 | Leaf-modifying | 2653 | 6010 |
| | | Tenthredinidae | sp. 1 | Free-feeding | 2 | 1 |
| | | Tenthredinidae | sp. 2 | Free-feeding | 19 | 3 |
| | | Tenthredinidae | sp. 3 | Free-feeding | 0 | 39 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Lepidoptera | Coleophoridae | Coleophoridae | sp. 1 | Leaf-modifying | 5 | 33 |
| | | Coleophoridae | sp. 2 | Leaf-modifying | 0 | 1 |
| | Erebidae | *Orgyia* | *leucostigma* | Free-feeding | 1 | 1 |
| | Gelechiidae | Gelechiidae | sp. 1 | Free-feeding | 0 | 1 |
| | Geometridae | *Campaea* | *perlata* | Free-feeding | 0 | 0 |
| | | Geometridae | sp. 1 | Free-feeding | 14 | 0 |
| | | Geometridae | sp. 2 | Free-feeding | 0 | 1 |
| | | Geometridae | sp. 3 | Free-feeding | 0 | 1 |
| | | Geometridae | sp. 4 | Free-feeding | 0 | 1 |
| | | Geometridae | sp. 5 | Free-feeding | 0 | 1 |
| | Gracilliaridae | *Caloptilia* | *stigmatella* | Leaf-modifying | 697 | 717 |
| | | *Phyllocnistis* | *populiella* | Leaf-modifying | 163 | 1436 |
| | | *Phyllonorycter* | *tremuloidiella* | Leaf-modifying | 1730 | 1902 |
| | Lyonetiidae | *Paraleucoptera* | *albella* | Leaf-modifying | 2 | 73 |
| | Nepticulidae | *Ectoedemia* | *populella* | Leaf-modifying | 2138 | 3696 |
| | | *Ectoedemia* | *argyropeza downesi* | Leaf-modifying | 3 | 0 |
| | Noctuidae | *Acronicta* | *lepusculina* | Free-feeding | 35 | 79 |
| | | *Catocala* | *relicta* | Free-feeding | 0 | 0 |
| | | *Ipimorpha* | *pleonectusa* | Free-feeding | 0 | 1 |
| | | *Orthosia* | *hibisci* | Free-feeding | 0 | 0 |
| | Notodontidae | *Gluphisia* | *septentrionis* | Free-feeding | 829 | 15 |
| | | *Pheosia* | *rimosa* | Free-feeding | 7 | 18 |
| | | *Clostera* | *albosigma* | Leaf-modifying | 852 | 69 |
| | Nymphalidae | *Limenitis* | *archippus* | Free-feeding | 14 | 3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | *Limenitis* | *arthemis* | Free-feeding | 14 | 3 |
| | Pyralidae | *Meroptera* | *pravella* | Leaf-modifying | 11 | 17 |
| | | Pyralidae | sp. 1 | Leaf-modifying | 1 | 2 |
| | | Pyralidae | sp. 2 | Leaf-modifying | 0 | 1 |
| | | Pyralidae | sp. 3 | Leaf-modifying | 0 | 1 |
| | | Pyralidae | sp. 4 | Leaf-modifying | 0 | 1 |
| | Saturniidae | *Actias* | *luna* | Free-feeding | 0 | 1 |
| | | *Hyalophora* | *cecropia* | Free-feeding | 0 | 2 |
| | Sphingidae | Sphingidae | sp. 1 | Free-feeding | 3 | 1 |
| | Tortricidae | *Choristoneura* | *rosaceana* | Leaf-modifying | 115 | 401 |
| | | Tortricidae | sp. 1 | Leaf-modifying | 5 | 5 |
| | Lepidoptera eggs | | | | 170 | 87 |
| | Diseased/parasitized Lepidoptera (unidentified) | | | | 9 | 36 |
| Orthoptera | Acrididae | Acrididae | sp. 1 | Free-feeding | 2 | 4 |
| Thysanoptera | Phlaeothripidae | Phlaeothripidae | sp. 1 | Free-feeding | 0 | 3 |
| **Total insects** | | | | | **50261** | **78838** |

**Supplemental Table 3.** Comparison of broad-sense heritabilities (with 95% confidence intervals) of various community metrics (Shannon index, richness, abundance) across different insect community data (all insect data, only common insect data, and only rare insect data) in 2014 and 2015. Common insect were found on >5% of the surveyed trees, while rare insects were found on <5% of trees. Rare insects exhibited negligible heritability.

| Data | Community trait | $H^2$ 2014 | $H^2$ 2015 |
|---|---|---|---|
| All insects | Shannon index | 0.08 (0.01 – 0.14) | 0.08 (0.02 – 0.15) |
| | Richness | 0.13 (0.06 – 0.19) | 0.11 (0.05 – 0.18) |
| | Abundance | 0.09 (0.03 – 0.15) | 0.10 (0.04 – 0.17) |
| Common species | Shannon index | 0.09 (0.02 – 0.15) | 0.09 (0.02 – 0.15) |
| | Richness | 0.13 (0.07 – 0.20) | 0.11 (0.04 – 0.17) |
| | Abundance | 0.11 (0.03 – 0.16) | 0.10 (0.04 – 0.17) |
| Rare species | Shannon index | 0.02 (-0.04 – 0.05) | 0.03 (-0.02 – 0.10) |
| | Richness | 0.01 (-0.04 – 0.04) | 0.06 (-0.01 – 0.12) |
| | Abundance | 0.00 (-0.04 – 0.03) | 0.11 (0.05 – 0.18) |

**Supplemental Figure 1.** This diagram outlines the (1) data structure for multilevel community models, (2) differences between fixed and mixed effects in this context, and (3) steps for fitting multilevel community models. The mixed-model approach can assess differences in composition and richness of the insect communities, but not abundance and evenness since the data are in presence/absence format. Thus, this method is a complement to the univariate community metric models, which take into account differences in community evenness (Shannon index) and abundance.

Standardized tree traits | Presence/Absence

| Tree ID | SLA | … | Foliar N | Species | Pres |
|---|---|---|---|---|---|
| 1 | 0.15 | … | -0.54 | A | 1 |
| 2 | -0.10 | … | 0.62 | A | 0 |
| 3 | 0.82 | … | 0.01 | A | 1 |
| 1 | 0.15 | … | -0.54 | B | 0 |
| 2 | -0.10 | … | 0.62 | B | 1 |
| 3 | 0.82 | … | 0.01 | B | 1 |
| 1 | 0.15 | … | -0.54 | C | 1 |
| 2 | -0.10 | … | 0.62 | C | 0 |
| 3 | 0.82 | … | 0.01 | C | 0 |

**Fixed effects** = tree traits that structure the entire community

- These effects are modeled as both linear (^1 power) and quadratic (^2 power) effects to identify potentially non-linear relationships among the insect community and tree traits

**Random effects** = tree traits that influence different insect species differentially

- These are modeled as only linear effects (^1 power)
- Random within the species grouping factor, *e.g.*, (0 + FoliarN|Species)

(1) Fit the null model [***Pres = (1|Species)***] using glmer (lme4 package in R, Bates et al. 2015) and use forward selection to identify random effects that should be included in the model
   - Note: the null model allows for different intercepts for each insect species

(2) Fit model with selected random effects and all fixed effects (linear + quadratic) to identify which fixed trait effects are significantly associated with the insect community, given the random effects

(3) Drop all non-significant fixed effects, but:
   - If a quadratic trait (*e.g.,* $SLA^2$) is significant, than the linear form (SLA) also must be included regardless of its significance (following hierarchical model rules)
   - If a random effect for a trait is included (*e.g.,* (0 + SLA|Species), then its corresponding linear fixed effect (SLA) must also be included even if it is insignificant (following Jackson et al. 2012)

"…" indicates that more standardized tree traits are included in analyses than shown in the above diagram

# Chapter Three

## Linking plant genes to insect communities: identifying the genetic bases of plant traits and community composition

Chapter Three is currently in preparation for submission to Molecular Ecology.

## Introduction

Identification of the genetic mechanisms of species interactions and community composition is a major aim of community genetics. Studies in this discipline have determined that different genotypes of plants have different communities of associated organisms (*e.g.,* insects, endophytes, etc.), and that community relatedness is mirrored by the genetic relatedness among plant genotypes in a common environment (Barbour et al. 2016, Keith et al. 2017, Koricheva and Hayes 2018, Kagiya et al. 2018). These associated communities are shaped by key plant traits, including morphology, phenology, and phytochemistry (Wimp et al. 2007, Robinson et al. 2012, Barbour et al. 2015, Barker et al. 2018). To date, this research has been conducted primarily at the plant genotype level and thus the identity of the underlying plant genes has remained largely unresolved.

Previous studies that have investigated the genetics of plant resistance to insect herbivores have assessed insect-associated damage, fitness, abundance and diversity traits (Rönnberg-Wästljung et al. 2006, Dewoody et al. 2013, Bernhardsson et al. 2013, Zinkgraf et al. 2016, Thoen et al. 2017). While many studies have explored the genetic basis of resistance in crops (*e.g.,* Tzin et al. 2015) and *Arabidopsis thaliana* (*e.g.,* Thoen et al. 2016), few have investigated the underlying plant genetics of complex insect communities (Dewoody et al. 2013, Bernhardsson et al. 2013). Dewoody *et al.* (2013) used QTL-mapping to identify genetic regions associated with foliar damage caused by various insect guilds in $F_2$ pedigrees of *P. trichocarpa x deltoides* hybrids. Bernhardsson and colleagues (2013) used association mapping to determine whether seven defense-related genes in European aspen (*Populus tremula)* correlated with insect guilds and

species richness. Both studies found a modest number of QTL (12-14) with small- to medium-sized effects ($R^2$ = 0.03-0.15 per QTL).

To significantly advance the field of community genetics, a broader experimental approach is required. First, research is needed at the genomic scale, with fine resolution (*e.g.,* genome-wide association mapping [GWAS], Ingvarsson and Street 2011), to pinpoint causative plant genes and genetic regions that structure associated communities. Second, like the work by Bernhardsson et al. (2013), plant traits (*e.g.,* metabolites) that are known to shape associated communities should be included in analyses to identify genes underlying the traits and to assess whether there is overlap with genes that underlie community composition. Third, more complex association models (*e.g.,* multivariate GWAS) can be used to better capture the complexity of community traits rather than simplifying these metrics to abundance and richness values.

Here, I identified genes underlying both ecologically relevant tree traits in, and associated insect communities on, trembling aspen (*Populus tremuloides*). I used a recently established genetic mapping population of 445 aspen genets. I quantified 20 tree traits, including size, growth, foliar morphology, foliar phenology, and phytochemistry, and surveyed herbivorous insect and ant communities. I then used both univariate and multivariate genome-wide association analyses with a dataset of over 170,000 SNPs (single nucleotide polymorphisms) for each aspen genet to identify the underlying genes. I predicted that some of the same loci that shaped tree traits would also be associated with the composition of insect communities.

## Methods

### *Study system*

Trembling aspen (*Populus tremuloides*) is the most widely distributed and genetically diverse tree species in North America (Mitton and Grant 1996). It exhibits little population structure, with evidence of only two subpopulations (southwestern and northern, Callahan et al. 2013) across its range. This feature makes aspen ideal for genome wide association analyses, since population structure can mislead results with false positive associations (Ingvarsson and Street 2011). In addition, aspen is a foundation species with substantial impacts on dependent communities (Hillstrom 2009). Studies of aspen (Hillstrom 2009, Barker et al. 2018) and other *Populus* species (Wimp et al. 2005, Bangert et al. 2006, Robinson et al. 2012) have linked plant genotypes to unique arthropod communities, and these communities have low to high broad-sense heritabilities (0.09 to 0.78 $H^2$). In addition, key tree traits (tree size, bud phenology, extra-floral nectaries, and secondary chemistry) have been shown to structure insect community composition (Bangert et al. 2006, Robinson et al. 2012, Barker et al. 2018).

### *WisAsp Common Garden*

The Lindroth Lab Group established a genetic mapping population of aspen in 2010 with genets collected from throughout Wisconsin (Fig. 1, latitude range: 358 km, longitude range: 186 km). The trees were planted in a randomized complete block design with four replicate blocks and a perimeter of non-experimental trees at the University of Wisconsin's Arlington Agricultural Research Station (SFig. 2). We originally

planted 392 genets, however due to vole damage many trees died in both 2010 (N = 399) and 2011 (N = 334). We thus replanted some of our original genets and also collected and planted new genets for a total of 445 aspen genets with 3.56 replicates on average (+/- 1.80 SD, N = 1824 in total). The garden was planted in a former grass-alfalfa field with Joy silt loam soil (USDA). Trees were planted with 2.5 m x 2.5 m spacing. The entire garden was surrounded by a 2.4 m tall electric fence to exclude deer, and the site was mowed and maintained as needed. The trees were 4-5 years old at the time of data collection (75% of the trees were at least 1.6 m and 2.6 m tall in 2014 and 2015, respectively).

*Tree Trait Surveys*

I measured ecologically relevant tree traits, including growth (measured 2012-2015), foliar morphology (2014-2015), phenology (2014-2015), and defense (phytochemistry 2014-2015, extra-floral nectaries 2014). To survey growth, I measured tree volume after each growing season. I recorded basal diameter (10 cm above ground level) and height (ground level to the base of the apical bud). Volume was calculated as $diameter^2 \times height$, a metric that correlates well with biomass (Stevens et al. 2007). I calculated absolute growth as $log_{10}(treevolume_{final} - treevolume_{initial})$ and relative growth as $ln(treevolume_{final}) - ln(treevolume_{initial})$, respectively.

To measure foliar morphology, I haphazardly collected 20-30 leaves from each tree in late June/early July and scanned them on a LICOR flatbed scanner (Version 3100, Lincoln, NE). The leaves were then vacuum-dried and weighed. I calculated both average individual leaf area and specific leaf area ($leaf area\ (cm^2)/mass\ (g)$).

To assess phenology, I recorded timing of bud break and bud set using 5-point and 2-point scales, respectively. The bud break scale was adapted from Robinson et al. (2012) and varied from (1) dormant buds to (3) broken buds to (5) leaves that were flushed and completely unrolled but not yet fully expanded (Supplemental Fig. 1). The bud set scale measured whether the buds were still growing (0) or set and dormant (1). I examined each tree every 2-3 days for each survey. For bud break I measured the most advanced bud on the tree, following Project BudBurst (http://budburst.org/) protocols, while for bud set I measured the terminal stem bud only. In addition to measuring date of bud break and bud set, I also calculated the length of the growing season for each tree in number of days as $Julian\ bud\ set\ date - Julian\ bud\ break\ date$.

To quantify foliar defenses and nitrogen levels, I collected leaves in both late June/early July (same leaves as were used for foliar morphology assessment) and August to analyze phytochemistry and EFN density, respectively. For the June/July collection, I pulverized the dried and weighed leaves to a fine powder by ball milling, and stored them at -20 $^{o}$C. I quantified foliar concentrations of nitrogen, condensed tannins, and phenolic glycosides using near infrared reflectance spectroscopy (NIRS; FOSS NIRSystems, Laurel, MD, USA), as described by Rubert-Nason et al. (2013). Spectra were collected from dry, powdered leaf samples packed into 5-cm ring cup cells. After exclusion of outlier spectra with a global Mahalanobis distance (MD) > 3, I developed calibrations relating NIR spectral bands (1100–2500 nm) to phytochemical parameters using a subset of samples (~150 – 400) chosen by the SELECT algorithm (WinISI v1.50 software Foss-Tecator, Infrasoft International LLC, State College, PA, USA) with a neighborhood MD of 1.0 (Shenk and Westerhaus 1991a, 1991b). I acquired

nitrogen reference values by combustion gas chromatography on a Thermo Flash EA1112 elemental analyzer (Thermo Finnigan, Milan, Italy), as described in Sollins et al. (1999). Condensed tannin reference values were measured colorimetrically (550 nm) relative to purified *P. tremuloides* condensed tannin material (Hagerman and Butler 1980), after extraction of foliage into 70:30 v/v acetone/water and reaction with Fe(III) under acidic conditions (Porter et al. 1986). Phenolic glycoside reference values were determined by extraction of foliage into methanol, followed by separation of extracts by ultra-high performance liquid chromatography and quantification by negative electrospray ionization single quadrupole mass spectrometry (Waters ACQUITY iClass UPLC/MS system, Milford, MA, USA), following methods adapted from Abreu et al. (2011) and Rubert-Nason et al. (2014). Samples with anomalous spectra (N~50, identified by MD>3) were also analyzed by these reference methods. I developed partial least squares regression models relating NIR spectra to phytochemical reference values (Table 1), and applied these models to predict the phytochemistry in all 1824 leaf samples from their corresponding NIR spectra.

To determine EFN density (number per leaf), I haphazardly collected 12 leaves from each tree in August 2014 and stored them on ice in the field. I then digitally scanned the upper surface of each leaf, and counted the number of EFNs that were present on each leaf in the scanned images. EFNs in aspen are located at the leaf/petiole juncture.

*Insect Community Surveys*

I visually surveyed herbivorous insect and ant species on the originally planted trees (N = 989, 328 aspen genets, all planted in 2010) in mid-July to early August 2014-15. I standardized my surveys by time to account for variation in tree canopy sizes. To ensure that my methods captured the complete insect community, I first constructed rarefaction curves of number of insect species observed per time interval (30 sec intervals with a total of 20 min surveyed for each tree) for 20 trees distributed throughout the garden (early July 2014 and2015). The rarefaction curves revealed that species richness saturated at approximately 3 min of survey time for small trees (< 1.5 m tall) and 6 min for large trees (>2.5 m tall). I therefore surveyed each tree for at least three minutes and larger trees were surveyed for additional time units (3 min increments). I then standardized the insect counts by minutes surveyed (*e.g.,* number of insects per species per minute). I stopped the survey time to collect, identify, and record insects.

In 2014, I surveyed the entire tree. In 2015, due to increased tree size, I divided the canopy into lower, middle, and upper sections and surveyed each for the same interval of time. I limited the maximum time interval to 4 min (12 min of survey time for a tree), which allowed us to survey a large subset of the canopy, but not necessarily the entire canopy. I surveyed the insect communities from 8:30AM to 4PM each day, and I conducted the surveys only on days with fine weather ($25.6℃ \pm 7.1$ average maximum temperature, $2.4\ \mathrm{m/s} \pm 1.2$ average wind speed, sunny to partly cloudy).

I trained a team of 6-7 surveyors for each insect survey (2014-15), and was present each day of the survey to address insect identification questions. Insects were identified using field guides and keys, and specimens were collected as needed for

further identification in the lab. All common insects were identified to species and rare insects to morpho-species (family-level). I also surveyed insect-inflicted damage, including leaf mines, leaf and petiole galls, and leaf rolls and tents. Vouchers of common insect species are preserved in the UW-Madison Wisconsin Research Insect Collection, and I have provided a website (https://aspeninsects.wordpress.com/) that displays information (life history, key identification features, etc.) for the common insect species found at WisAsp.

*Genetic Analyses*

Foliar DNA was extracted from one ramet in each genet (leaves were collected in June 2012-14 and freeze-dried prior to extraction). Probes (N = 65,000, 150 bp in length) were designed by Rapid Genomics (Gainesville, FL) and Nathaniel Street (Umeå Plant Science Center, Sweden) to align to each gene (exome capture) in the *P. tremula* scaffold genetic map, which includes 9,789 scaffolds covering 36,322 genes (Sjödin et al. 2009). These probes were then tested on a population of 24 *Populus* genets to identify probes that were suitable for sequencing (*e.g.,* mapped to a unique gene region). This test resulted in 45,934 probes on 5,478 scaffolds and located in 20,483 genes, with an average of 2.3 probes/gene for sequencing. Extracted DNA was then sequenced with these 45,935 probes with a minimum sequencing depth of 15x per sample. Based on technical replicates, the sequencing error rate was 0.2%. These sequences were then aligned to the *P. tremula* scaffold map and single nucleotide polymorphisms (SNPs) were identified by Dr. Carolina Bernhardsson (Swedish University of Agricultural Sciences, Sweden) using GATK HaplotypeCaller.

A subset of genets (N = 11) had been sequenced previously with whole-genome sequencing (for complete details, see Wang et al. 2016). SNPs from these 11 genets were merged with SNPs from probe sequencing (434 genets) and filtered for genotype and sample quality metrics using VCF and BCFtools (Danecek et al. 2011, see full SNP filtering pipeline in STable 2). After the SNPs were filtered, missing genotype information was imputed using LinkImpute (using default settings, Money et al. 2015). This SNP filtering pipeline resulted in a dataset of 173,520 SNPs distributed throughout the *P. tremula* genome on 5,332 scaffolds and 20,483 genes with eight SNPs per gene on average.

*Statistical Methods*

Several factors can influence the success of genome-wide association (GWA) analyses to detect significant genes underlying traits of interest (Ingvarsson and Street 2011), including measurement error and population structure. Measurement error can bias the trait data, impacting the ability to find SNP associations, whereas underlying genetic structure can lead to the detection of spurious SNP associations. To deal with potential biases, I first assessed structure within the WisAsp population using the multi-locus approach, Admixture (Alexander et al. 2009). The filtered SNPs were first pruned by linkage disequilibrium before analyses (pairs of SNPs within a 50 bp window were pruned if they exhibited an $r^2$ value greater than 0.2 using PLINK), resulting in 139,338 SNPs. This set of SNPs was then analyzed with various population structures (K = 1 through 5) and cross-validation to identify the number of populations that best explain variation in allele frequencies among genets.

I then performed both univariate and multivariate GWA analyses to identify genetic regions in aspen that are associated with phenotypic traits (*i.e.,* phenology, growth, leaf morphology, phytochemistry, and extra floral nectaries) and insect populations (*i.e.,* presence or abundance of particular species), families, guilds, and community metrics (*i.e.,* species richness, abundance, nonmetric multidimensional scaling [NMDS] axes for Bray-Curtis community dissimilarity matrices; for complete list of traits see STable 3). I first regressed each trait and metric (*e.g.,* species richness) on covariates, including experimental block and year in which the data were collected (both as fixed effects) and genet (as a random effect) using lme4 in R (Bates et al. 2015). From these models, I extracted the best linear unbiased predictors (BLUP) for each genet and rank-transformed these values for GWA analyses.

To both identify gene associations and conduct sensitivity analysis for my GWA models, I used two statistical packages: PLINK v.1.9 (Purcell et al. 2007) for both univariate and multivariate traits and Genome-wide Efficient Mixed Model Association (GEMMA v.0.96, Zhou and Stephens 2012) for univariate traits (results shown in supplemental data). PLINK uses a simple linear regression without corrections for relatedness among individuals, while GEMMA uses a compressed mixed linear model (Zhang et al. 2010), which controls for relatedness among individuals with a centered kinship matrix. Kinship did not appear to be an important factor for my WisAsp population, since inclusion of a kinship matrix in GWA analyses had little effect on the results (90% of the significant associations remained, STable 4). I did not include any covariates for population structure, since Admixture results indicated that my sample population is panmictic (Fig. 1). For data analyses, I used SNPs with a minor allele

frequency of at least 0.05 and I corrected for multiple testing with a false discovery rate (FDR) of 0.10 (which is similar to previous FDR cut-off values for Salicaceae GWAS studies, *e.g.,* Hallingbäck et al. 2016) using the qvalue package in R (Bass et al. 2015). In addition, I pruned full-siblings (seven genets were removed) from my dataset for the GWA analyses. After pruning these genets, genetic relatedness was extremely low within the WisAsp population, with a mean of -0.002 (± 0.007 SD, Fig. 1).

To further elucidate the function of particular genetic regions and the mechanism by which they may influence insects, I compared significant associations with the annotated *P. trichocarpa* genome (Tuskan et al. 2006), *P. tremuloides* genetic map (Ingvarsson unpublished data) and *Arabidopsis thaliana* genome (Swarbreck et al. 2007) using the *Populus* Genome Integrative Explorer (PopGenIE, Sjödin et al. 2009). To determine whether particular tree traits were important in structuring insect communities, I included various standardized tree traits as covariates in the insect community GWA analyses in PLINK. If the significant insect-associated SNPs disappeared with inclusion of the tree trait covariates, I inferred that these tree traits were important in shaping the SNP-related variation in the insect phenotype.

To link gene functions, products, and processes to associated traits, I conducted gene set enrichment analysis using the generic gene ontology mapper (http://go.princeton.edu/cgi-bin/GOTermMapper, Gene Ontology Consortium et al. 2004, Boyle et al. 2004), including the top 0.1% most significant SNPs (N = 174) from each GWA test. These gene sets were compared to the background set of genes that were included in my probe set (based on *Arabidopsis* homologs) and only unique gene

names were used to control for differences in gene size and the number of SNPs/gene. These tests were run with a Bonferroni p-value cutoff of 0.10.

**Results**

Of 79 GWAS tests conducted for various tree and insect traits, 15 resulted in significant associations (Table 1 and STable 3). Of these, 49 SNPs (nine synonymous and 40 nonsynonymous) in 13 different genes were identified, which were distributed across eight pseudo-chromosomes (based on *P. trichocarpa* v3.0 genome alignment). Individual SNPs explained 5.7-8.4% of the phenotypic variation for the associated trait ($R^2$ values in Table 1). Many of the significant genes are involved in gene expression, protein modification, and the movement of resources in and out of cells. In addition, several of the identified genes are known to be regulated by plant hormones (jasmonic acid, abscisic acid, brassinosteroids, ethylene).

*SNPs associated with tree trait variation*

Of the 20 univariate GWA tests for tree traits, three resulted in significant associations. These associations were for levels of tremulacin, phenolic glycosides (combined levels of tremulacin and salicortin) and total defense phytochemistry (combined levels of tremulacin, salicortin, and condensed tannins, Table 1). Two SNPs found in *Potra003979g23949* that encode for an ASC1-like (Cyp1 Absence of growth Suppressor) protein were identified in GWA analyses for both tremulacin and phenolic glycosides. The associated genes accounted for 5.9%, 5.9%, and 5.7% of the variation in tremulacin, phenolic glycosides, and total defense phytochemistry, respectively. No

SNPs were identified for tree growth/size metrics, bud phenology, leaf morphology, foliar nitrogen, and the density of extra floral nectaries (EFN). Multivariate GWA analysis of uncorrelated ($r < 0.70$) tree traits (*i.e.*, relative and absolute growth, specific leaf area, individual leaf area, EFN density, growing season length, and levels of condensed tannins, phenolic glycosides, and nitrogen) also resulted in no significant SNPs.

Of the 20 gene set enrichment tests for tree traits, eight resulted in significantly enriched gene ontology terms for variation in tree size/growth metrics (e.g., spring volume, basal area increment), specific leaf area, and EFN density (Table 2). Many of the tree size/growth metrics were enriched for genes involved in response to misfolded proteins. Specific leaf area was enriched for genes involved in defense (immune) response. EFN density was enriched for genes involved in glyceraldehyde-3-phosphate metabolism.

*SNPs associated with insect variation*

Of the 49 GWA tests for insect univariate traits, ten resulted in significant associations. These associations were for variation in the incidence of *Ecteodemia populella* (petiole-galling moth), *Phyllonorycter tremuloidiella* (blotch mining moth), *Clostera albosigma* (leaf-rolling moth), Cecidomyiidae (leaf-rolling flies), and *Lasius neoniger* (most common ant species), and the abundance of *Clostera albosigma*, *Choristoneura rosaceana* (leaf-rolling moth), Tortricidae (leaf-rolling moths), and *Lasius neoniger* (Table 1). The significantly associated genes accounted for 7.4-22.6% of the total variation for the insect traits (summing across all genes identified for each insect trait). No SNPs were identified for free-feeding insects, leaf-galling flies, several leaf-

mining moth species, and insect community metrics (*i.e.,* abundance, richness, Shannon index, STable 3).

To further explore the importance of particular tree traits to insect phenotypes, I conducted GWA analyses with those traits incorporated as covariates. Loss of significant SNP associations would indicate that the tree trait was important in (directly or indirectly) shaping the gene-insect relationship. Including tree trait covariates in the GWA models made many of these significant insect associations disappear (Table 3). In particular, individual leaf area, defense phytochemistry, and tree size/growth traits eliminated all of the significant associations for particular leaf-modifying insects. In addition, significant associations for leaf-rolling insect species disappeared when bud phenology was included in the analysis. No tree trait covariate could completely eliminate the 12 significant SNPs for *Lasius neoniger* abundance or incidence, but inclusion of either individual (tremulacin and salicortin) or combined levels of phenolic glycosides made 3-4 of the SNPs (found in *Potra002557g19270* and *Potra003286g21239*) insignificant.

Of the nine GWA tests for insect multivariate traits, two resulted in significant associations. These associations were for nonmetric multidimensional scaling (NMDS) axes for variation in both the abundance and presence/absence of common insect species (Fig. 2). Both multivariate traits were associated with the same gene, *Potra001060g09097.* The significant SNPs had variable effects on the different components of the trait, with coefficients ranging from -0.72 to 0.82 (Fig. 2 C). Specifically, aspen with the C allele at these SNP sites had a more diverse insect community (all common insects were more often present on these trees; Fig. 2 B) that

was dominated by a few very abundant species (*Harmandia* sp., aphids, and aphid-tending ants; Fig. 2 A).

Of the 58 gene set enrichment tests for insect phenotypes, 14 resulted in significantly enriched gene ontology terms (Table 2). Several of the enriched gene sets were associated with leaf-galling and -rolling insects and multivariate insect traits. The enriched gene ontology terms included biosynthesis of an anthocyanin-containing compound, response to mechanical stimulus, cell wall biogenesis (notably for a leaf-galling insect species), γ-aminobutyric acid (GABA) transport, and hormone biosynthesis/regulation (Table 2).

**Discussion**

Community genetics research has highlighted the importance of plant intraspecific variation in structuring associated communities (Schweitzer et al. 2008, Barbour et al. 2016, Keith et al. 2017, Koricheva and Hayes 2018, Kagiya et al. 2018). That work, however, has focused primarily at the plant genotype level, and thus the identity of the causative genes remains largely unresolved. Previous studies identified a limited set of *Populus* genes associated with insect damage (*e.g.,* mines, galls, herbivory, etc.; Dewoody et al. 2013, Zinkgraf et al. 2016) and insect community metrics (*e.g.,* abundance, richness; Bernhardsson et al. 2013). My research advances the discipline by identifying both genes and gene functions that underlie ecologically relevant tree traits and insect communities, and exploring relationships between insect gene associations and tree traits. Most notably, my research discovered a plant gene that underlies a complex community trait (*e.g.,* NMDS axes), which to my knowledge is

the first of its kind. My findings also reveal new gene associations for variation in defense compounds, including salicinods. In addition, I found ten new gene associations for variation in the abundance and incidence of leaf-modifying insects and ants. Third, my results show that the effects of many of the insect-associated genes are explained by variation in tree traits, including phytochemistry, individual leaf area, timing of bud break, and tree size. Finally, I identified gene functions and processes that are associated with tree size, growth, and leaf morphology, and leaf-galling, leaf-rolling, and free-feeding insects, ants, and insect community composition.

*Genes underlying tree traits*

My phytochemical traits had high heritability ($H^2 > 0.6$), which likely explains why the underlying genes were detectable (Beavis 1994). Both tremulacin and total phenolic glycosides (combined tremulacin and salicortin levels) were associated with an ASC1-like protein, which is involved in protein translation and potential regulation by abscisic acid (Guo et al. 2011). In addition, total defense phytochemistry (combined condensed tannin and phenolic glycoside levels) was associated with a ribosomal protein (*Potra007960g26067*) that is near a syntaxin protein (*SYP121*). This syntaxin protein is regulated by various hormones, including jasmonic acid, abscisic acid, and salicylic acid, and the protein is involved in programed cell death and defense response to pathogens. Thus, these newly identified defense-related genes are both involved in gene expression and are regulated by plant hormones. These findings shed light on the phytochemical pathway for salicinoids, which remains largely unresolved (Boeckler et al. 2011).

In addition to gene associations, a few tree traits were associated with enriched gene functions. Tree growth and size traits were enriched for cellular response to misfolded proteins, which is essentially a response to environmental stress (*e.g.,* heat, cold, UV; Nakajima and Suzuki 2013). Specific leaf area was enriched for defense ("immune") response, and the density of extra floral nectaries was enriched for glyceraldehyde-3-phosphate metabolism, which is involved in glycolysis.

*Genes underlying insect communities*

Upon insect herbivory, plants experience damage-induced ion imbalances, which leads to differing cell membrane potentials, calcium signaling, and oxidative stress (Maffei et al. 2007). These events alter kinase and phytohormone activity, which then influences gene expression (Maffei et al. 2007). My insect community GWAS and gene ontology analyses revealed candidate genes with functions that are consistent with this series of plant-insect events.

First, variation in Tenthredinidae sawflies (primarily leaf-folding *Phyllocolpa* sp.) was associated with genes enriched for sequestering iron ions (that have also been shown to respond to reactive oxygen species [Ravet et al. 2009]) perhaps due to damage-induced ion imbalances. Second, variation in leaf blotch miner (*Phyllonorycter tremuloidiella*) incidence was correlated with a vesicle transport protein (*Potra000892g07232*), which is physically located near a calmodulin-binding NAC protein (*NTL9*) and may influence calcium signaling. Third, variation in ant (*Lasius neoniger*) incidence and abundance was related to a tocopherol gene (*Potra002557g19270*), which responds to oxidative stress (*i.e.,* tocopherol [vitamin E]

inhibits the breakdown of fatty acids in plant cell membranes by eliminating reactive oxygen species, Porfirova et al. 2002). Fourth, variation in insects (incidence and composition) was related to genes involved in plant hormone regulation. For instance, petiole-galler (*Ecteodemia populella*) incidence was related to an abscisic acid receptor (*Potra001062g09110*), and insect community composition was enriched for gene ontology terms involved in hormone regulation. Fifth, both petiole-gallers and ants were associated with genes involved in modifying expression of other genes, including *Potra001062g09111* (transcriptional silencing via DNA methylation), *Potra003266g21171* (mRNA splicing factor that responds to biotic stress, Shang et al. 2017), and *AT2G40435* (*A. thaliana* homolog, transcription factor SCREAM-like protein that is involved in response to environmental stress, Liu et al. 2007).

I identified both enriched gene functions and SNPs associated with leaf-galling insects. Nabity et al. (2013) compared gene expression patterns in leaf gall tissue (*Daktulosphaira vitifoliae* galls on grape leaves) to those of regular leaf tissue. They revealed that leaf galls had upregulated the phenylpropanoid pathway, increased anthocyanin production and cell wall biogenesis, changed the expression of glycolysis/cellular respiration, and down-regulated the Calvin cycle. These findings are consistent with my gene enrichment analysis; genes associated with leaf-galling insects were enriched for flavonoid and anthocyanin biosynthesis, cell wall biogenesis, cellular respiration, and the tricarboxylic acid cycle. Also, both the *Harmandia* leaf galls and *Ecteodemia populella* petiole galls on my trees were strongly colored with red/purple-pigment, suggesting the presence of anthocyanins. In addition, I identified an apoptosis-inducing factor, *Potra002833g20082*, that was associated with Cecidomyiidae (leaf-

galling *Harmandia* flies and leaf-rolling midges), which may confer resistance to gall formation.

I also identified both enriched gene functions and SNPs associated with leaf-rolling insects. Variation in leaf-rolling insect (Tortricidae) abundance was related to genes involved in mechanical stimulus response and defense. For example, variation in rustylined leaf-tiers (*Clostera albosigma*, Notodontidae) was correlated with a glycerol kinase (*A. thaliana* homolog, *AT4G38225*), which is involved in glycolysis. Glycolysis produces precursors for the shikimic acid pathway, which synthesizes secondary plant compounds such as condensed tannins and phenolic glycosides. In addition, variation in obliquebanded leafroller (*Choristoneura rosaceana,* Tortricidae) abundance was associated with *NOXY2* (*NONRESPONDING TO OXYLIPINS 2, AT5G11630*), a salicylic acid responsive gene that has been shown to play a role in defense against bacterial pathogens (Vellosillo et al. 2013).

Both insect community composition and variation in ant (Formicidae) abundance and incidence were associated with Gamma-aminobutyric acid (GABA) signaling (via enriched gene ontology). GABA signaling influences plant growth, development, stress response, and long-distance transport (Ramesh et al. 2017). In addition, GABA signaling plays a role in insect resistance (Bown et al. 2006, Scholz et al. 2015) by causing physiological stress in the insect that decreases its growth and survival (Ramesh et al. 2017).

Insect community composition (NMDS of common insect species) was also associated with an early nodulin-like (ENODL) transmembrane protein (*Potra001060g09097)*, that is thought to transport carbohydrates (Denancé et al. 2014).

Wang et al. (2015) identified three ENODL proteins that putatively increased Bt rice resistance to brown planthopper infestation, thereby suggesting that ENODL proteins may influence plant-insect interactions. Here, I show that allelic variation in an ENODL gene influences insect community species diversity and the abundance of interacting foundation species: aphids and tending ants (Keith et al. 2010, Wimp and Whitham 2012, Lamit et al. 2015, Barker et al. 2018). To my knowledge, this is the first identification of a plant gene that is associated with a complex community trait (*i.e.,* NMDS of insect community composition).

*Plant trait variation shapes insect communities*

While I found no overlap in SNP associations across tree traits and insect phenotypes (potentially due to the limited number of tree trait-associated SNPs), my covariate analyses revealed that several tree traits explained the significant insect-SNP associations. These results suggest that the tree traits are important in structuring the associated insect communities, thereby providing a mechanistic link by which plant genes shape insect community composition (*e.g.,* Bangert et al. 2006). Previous work in *Populus* and *Salix* has also shown that canopy insect communities are shaped by these particular plant traits, including plant size (Robinson et al. 2012, Barbour et al. 2015, Evans et al. 2016, Barker et al. 2018), individual leaf area (Robinson et al. 2012), timing of bud break (Evans et al. 2016, Falk 2017, Barker et al. 2018), and defense phytochemistry (Wimp et al. 2007, Barbour et al. 2015, Brito 2017, Barker et al. 2018). For instance, larger plants and plants with larger leaves and longer petioles have denser and more diverse insect communities (Robinson et al. 2012, Barker et al. 2018).

Timing of bud phenology *differentially* affects the incidence of leaf-modifying insect species (Barker et al. 2018). Also, variation in defense phytochemistry corresponds with variation in insect communities (Bangert et al. 2006, Wimp et al. 2007).

Previously identified *Populus* genes that correlate with insect metrics have been linked to plant traits, including defense and leaf morphology. Nine of the 13 insect-associated QTL found by Dewoody et al. (2013) contained shikimate-phenylpropanoid pathway genes (both phenolic glycosides and condensed tannins are products of that pathway), and two of the QTL were in genomic hot-spots for leaf morphology. In addition, Bernhardsson et al. (2013) found overlap between insect-associated SNPs and inducible defense genes (polyphenol oxidases and trypsin inhibitors).

Inclusion of tree trait covariates in my GWA models did not eliminate all of the ant-related genes (*i.e.,* ant-SNP associations were not fully explained by tree trait variation): the SNPs in the mRNA splicing factor (*Potra003266g21171)* that responds to biotic stress remain. This implies that (1) tree traits that I did not survey or (2) more likely, biotic/environmental factors (*e.g.,* aphid populations; most of my ant species were tending aphid colonies) structure ant incidence and abundance. In addition, this finding indicates that *Potra003266g21171* (Thioredoxin-like protein) is involved in phenotypic expression of tree traits other than tree growth/size, bud phenology, leaf morphology, and phytochemistry.

While this research focused on bottom-up mechanisms underlying insect communities on aspen, I recognize that top-down factors (*e.g.,* predation, parasitism, etc.) also influence insect herbivores (Frank van Veen et al. 2006, Katano et al. 2015, Vidal and Murphy 2018). A meta-analysis by Vidal and Murphy (2017) revealed that top-

down forces are often stronger than bottom-up effects in influencing insect herbivore fitness. Yet, these differences varied across insect groups. Specialist insects were affected more by bottom-up than top-down factors, while the opposite was true for generalist insects. In addition, chewing, sucking, and galling insects were affected more by top-down forces, while there was no difference in bottom-up vs. top-down effects for leaf-mining insect herbivores (Vidal and Murphy 2017). Thus, my limited association of plant genes to insect metrics likely derived in part from the effects of other ecological interactions on structuring these insect communities.

*Gene coverage*

My genetic dataset included 56% of the *P. tremuloides* genes, a coverage rate that reduced my ability to detect significant associations for some traits. To determine the extent to which my probe design included or excluded genes that are known to influence particular traits, I used Knetminer (Knowledge Network Miner, Hassani-Pak 2017) with *P. trichocarpa* homologs to compare my gene list to lists of genes that are associated with particular trait search terms (*e.g.,* “phenylpropanoid pathway”, “biomass”, “SLA”, “insect”, etc.). My gene list covered 54-71% of the genes associated with tree traits and insect resistance (STable 5). Although my probe set did not include every gene, my findings nonetheless reveal new gene associations that underlie both *Populus* traits and canopy insect communities. In comparison, previous *Populus* GWAS studies captured smaller sets of genes (1,233 to 18,153 genes) with fewer SNPs (1,233 to 77,000 SNPs; McKown et al. 2014, Du et al. 2015, Hallingbäck et al. 2016, Fahrenkrog et al. 2017).

*Conclusions*

Over the last 15 years, community genetics perspectives linking plant intraspecific genetic variation to associated community metrics have become established in the literature of evolutionary ecology. Most of this research, however, has been conducted at the level of plant genotypes, leaving the underlying genes unresolved. Here, I identified ten new *Populus* genes that structured associated insect communities, complementing the previously identified list of QTL from Bernhardsson et al. (12 SNPs, 2013) and DeWoody et al. (14 QTL, 2014). My findings also reveal that ecologically-relevant plant traits structure gene-insect associations, highlighting the importance of these traits as the mechanistic bridge between plant genes and insect communities (Robinson et al. 2012, Barbour et al. 2015, Barker et al. 2018, Harrison et al. 2018).

Genetic variation in expression of key plant traits is influenced by both plant ontogeny and environmental context (Lindroth and St. Clair 2013). Future work should address how plant genetic contributions to insect community organization may shift across plant ontogenetic trajectories (Holeski et al. 2012, Gosney et al. 2014) and environmental gradients (Burkle et al. 2013).

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**Table 1.** Summary of significant single nucleotide polymorphism (SNP) associations (false discovery rate [FDR] q-value < 0.10) for tree traits and insect phenotypes for the WisAsp *Populus tremuloides* genetic mapping population (N = 445 genets for tree traits, N = 328 genets for insect traits). Genome-wide association models were analyzed in PLINK without a kinship matrix. Bolded alleles are the minor allele.

| | | | | | | | | | | Homologous gene | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Trait** | ***P. tremula* (v1.1)** | **SNP position** | **Alleles** | | **MAF[‡]** | **P-value** | **FDR q-value** | **Beta (SE)[§]** | **$R^2$** | ***P. tremuloides* (v1.1)** | ***P. trichocarpa* (v3.0)** | ***A. thaliana*** | **Gene annotation** |
| Tremulacin | Potra003979g23949 | 37860 | **A** | T | 0.054 | 2.95E-07 | 0.025 | 0.792 (0.152) | 0.059 | Potrs002611g30592 | Potri.004G141000 | AT3G19260 | ASC1-like protein |
| | | 38233 | **G** | C | 0.054 | 2.95E-07 | 0.025 | 0.792 (0.152) | 0.059 | | | | |
| Phenolic glycosides* | Potra003979g23949 | 37860 | **A** | T | 0.054 | 2.50E-07 | 0.021 | 0.798 (0.152) | 0.059 | Potrs002611g30592 | Potri.004G141000 | AT3G19260 | ASC1-like protein |
| | | 38233 | **G** | C | 0.054 | 2.50E-07 | 0.021 | 0.798 (0.152) | 0.059 | | | | |
| Total defense chemistry** | Potra007960g26067 | 4119 | **A** | G | 0.462 | 3.93E-07 | 0.066 | -0.341 (0.066) | 0.057 | Potrs039467g25117 | Potri.001G218700 | AT3G52580 | 40S ribosomal protein |
| *Ecteodemia populella* (P/A)[†] | Potra001062g09110 | 18542 | **C** | T | 0.059 | 5.64E-07 | 0.062 | -0.887 (0.173) | 0.081 | Potrs042256g26529 | Potri.018G046100 | AT2G25770 | Abscisic acid receptor |
| | Potra001062g09111 | 28996 | **T** | A | 0.062 | 1.46E-06 | 0.062 | -0.835 (0.170) | 0.075 | Potrs007988g11232 | Potri.018G046200 | AT1G15215 | Protein SAWADEE HOMEODOMAIN HOMOLOG |
| | | 29108 | **A** | C | 0.062 | 1.46E-06 | 0.062 | -0.835 (0.170) | 0.075 | | | | |
| | | 29109 | **T** | A | 0.062 | 1.46E-06 | 0.062 | -0.835 (0.170) | 0.075 | | | | |
| | | 29512 | **T** | C | 0.054 | 1.85E-06 | 0.063 | -0.881 (0.081) | 0.074 | | | | |
| *Phyllonorycter tremuloidiella* (P/A)[†] | Potra000892g07232 | 177159 | **C** | T | 0.249 | 1.70E-06 | 0.098 | -0.459 (0.094) | 0.074 | Potrs009143g14209 | Potri.007G124400 | AT5G01430 | Vesicle transport protein |
| | | 177288 | **C** | T | 0.249 | 1.70E-06 | 0.098 | -0.459 (0.094) | 0.074 | | | | |
| | | 177289 | **A** | G | 0.249 | 1.70E-06 | 0.098 | -0.459 (0.094) | 0.074 | | | | |
| *Clostera albosigma* | Potra001567g12957 | 32991 | **T** | C | 0.241 | 4.53E-07 | 0.078 | -0.471 (0.091) | 0.082 | Potrs004442g06451 | Potri.004G207400 | AT4G38225 | Uncharacterized protein LOC103933534 isoform |
| *Clostera albosigma* (P/A)[†] | | 32991 | **T** | C | 0.241 | 5.63E-07 | 0.097 | 0.468 (0.091) | 0.081 | | | | |
| *Choristoneura rosaceana* | Potra000544g03858 | 259777 | **C** | T | 0.436 | 9.95E-07 | 0.098 | -0.460 (0.092) | 0.078 | Potrs019807g22433 | Potri.006G237800 | AT5G11630 | Uncharacterized protein LOC105122277 |
| | | 262984 | **A** | T | 0.435 | 1.13E-06 | 0.098 | -0.460 (0.093) | 0.077 | | | | |
| Cecidomyiidae | Potra002833g20082 | 5929 | **T** | C | 0.043 | 3.30E-07 | 0.056 | -1.076 (0.206) | 0.084 | Potrs005298g07564 | Potri.001G217800 | AT3G44190 | Apoptosis-inducing |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cecidomyiidae (P/A)† | | 5929 | **T** | C | 0.043 | 3.83E-07 | 0.066 | 1.069 (0.206) | 0.083 | | | | factor |
| Tortricidae | Potra000544g03858 | 259777 | **C** | T | 0.436 | 1.02E-06 | 0.100 | -0.460 (0.092) | 0.077 | Potrs019807g22433 | Potri.006G237800 | AT5G11630 | Uncharacterized protein LOC105122277 |
| | Potra000544g03859 | 262984 | **A** | T | 0.435 | 1.15E-06 | 0.100 | -0.460 (0.093) | 0.077 | Potrs042389g26631 | Potri.002G045500 | AT5G43680 | Sec-independent protein translocase protein TatB |
| *Lasius neoniger* | Potra003266g21171 | 16319 | **A** | T | 0.305 | 1.93E-06 | 0.042 | 0.387 (0.080) | 0.074 | Potrs017980g20216 | Potri.001G287600 | AT3G24730 | Thioredoxin-like protein |
| | | 16337 | **A** | G | 0.305 | 1.93E-06 | 0.042 | 0.387 (0.080) | 0.074 | | | | |
| | | 16338 | **G** | A | 0.305 | 2.75E-06 | 0.053 | 0.378 (0.079) | 0.071 | | | | |
| | | 16381 | **A** | G | 0.303 | 1.67E-06 | 0.042 | 0.391 (0.080) | 0.074 | | | | |
| | | 16473 | **T** | C | 0.303 | 1.67E-06 | 0.042 | 0.391 (0.080) | 0.074 | | | | |
| | | 16480 | **G** | A | 0.303 | 1.67E-06 | 0.042 | 0.391 (0.080) | 0.074 | | | | |
| | | 16548 | **G** | A | 0.300 | 1.35E-06 | 0.042 | 0.400 (0.081) | 0.076 | | | | |
| | | 16549 | **G** | A | 0.300 | 1.35E-06 | 0.042 | 0.400 (0.081) | 0.076 | | | | |
| | Potra002557g19270 | 32237 | **G** | A | 0.119 | 4.85E-06 | 0.070 | 0.537 (0.115) | 0.068 | Potrs004787g06948 | Potri.018G040200 | AT4G32770 | Tocopherol cyclase, chloroplastic |
| | | 32326 | **T** | C | 0.119 | 4.85E-06 | 0.070 | 0.537 (0.115) | 0.068 | | | | |
| | | 32327 | **C** | A | 0.119 | 4.85E-06 | 0.070 | 0.537 (0.115) | 0.068 | | | | |
| | Potra003286g21239 | 28246 | **T** | C | 0.071 | 9.33E-07 | 0.042 | -0.771 (0.154) | 0.078 | Potrs009219g14285 | Potri.019G126800 | AT2G40435 | Uncharacterized protein LOC105123201 |
| *Lasius neoniger* (P/A)† | Potra003266g21171 | 16319 | **A** | T | 0.305 | 1.27E-06 | 0.027 | -0.393 (0.079) | 0.076 | Potrs017980g20216 | Potri.001G287600 | AT3G24730 | Thioredoxin-like protein |
| | | 16337 | **A** | G | 0.305 | 1.27E-06 | 0.027 | -0.393 (0.079) | 0.076 | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 16338 | **G** | A | 0.305 | 1.82E-06 | 0.035 | -0.393 (0.079) | 0.074 | | | | |
| | 16381 | **A** | G | 0.303 | 1.15E-06 | 0.027 | -0.397 (0.080) | 0.077 | | | | |
| | 16473 | **T** | C | 0.303 | 1.15E-06 | 0.027 | -0.397 (0.080) | 0.077 | | | | |
| | 16480 | **G** | A | 0.303 | 1.15E-06 | 0.027 | -0.397 (0.080) | 0.077 | | | | |
| | 16548 | **G** | A | 0.300 | 9.17E-07 | 0.027 | -0.406 (0.081) | 0.078 | | | | |
| | 16549 | **G** | A | 0.300 | 9.17E-07 | 0.027 | -0.406 (0.081) | 0.078 | | | | |
| Potra002557g19270 | 32237 | **G** | A | 0.119 | 4.53E-06 | 0.066 | -0.538 (0.115) | 0.068 | Potrs004787g06948 | Potri.018G040200 | AT4G32770 | Tocopherol cyclase, chloroplastic |
| | 32326 | **T** | C | 0.119 | 4.53E-06 | 0.066 | -0.538 (0.115) | 0.068 | | | | |
| | 32327 | **C** | A | 0.119 | 4.53E-06 | 0.066 | -0.538 (0.115) | 0.068 | | | | |
| Potra003286g21239 | 28246 | **T** | C | 0.071 | 7.79E-07 | 0.027 | 0.776 (0.154) | 0.079 | Potrs009219g14285 | Potri.019G126800 | AT2G40435 | Uncharacterized protein LOC105123201 |

* Phenolic glycosides = combined levels of salicortin and tremulacin

** Total defense chemistry = combined levels of salicortin, tremulacin, and condensed tannins

† P/A = presence/absence

‡ MAF = minor allele frequency

§ SE = standard error

**Table 2.** Summary of WisAsp gene set enrichment analyses for tree traits and insect phenotypes. Analyses were conducted on gene lists (top 0.1% most significant SNPs, N = 174 SNPs) from PLINK genome-wide association results. Gene sets were compared to the background set of genes that were included in my probe set (based on *Arabidopsis* homologs).

| Trait category | Trait | Enriched gene ontology term | Gene set frequency | Background frequency | Bonferroni p-value | FDR[§] q-value | *Arabidopsis thaliana* homologous genes |
|---|---|---|---|---|---|---|---|
| Tree size/ growth | Spring volume | Cellular response to misfolded protein | 1.9 | 0.0 | 0.07 | 0.12 | AT4G03510, AT5G52060 |
| | Absolute growth | | 1.9 | 0.0 | 0.07 | 0.48 | |
| | Spring basal area | | 1.8 | 0.0 | 0.06 | 0.36 | |
| | Average basal area | | 1.8 | 0.0 | 0.09 | 0.18 | |
| | Average volume | | 1.8 | 0.0 | 0.07 | 0.22 | |
| | Basal area increment | Sucrose metabolic process | 3.5 | 0.2 | 0.01 | 0.02 | AT1G22710, AT5G40390, AT3G03250, AT3G22200 |
| Leaf morphology | Specific leaf area | Immune response process | 8.3 | 1.8 | 0.04 | 0.06 | AT1G42990, AT5G65600, AT5G64930, AT4G24290,AT5G64570, AT3G04740, AT2G39200, AT3G50930, AT5G18610, AT4G37930 |
| | | Innate immune response | 8.3 | 1.5 | 0.01 | 0.02 | AT5G65600, AT5G64930, AT4G24290, AT5G64570,AT3G04740, AT2G39200, AT3G50930, AT5G18610, AT4G37930 |
| | | Immune response | 8.3 | 1.5 | 0.04 | 0.02 | AT5G65600, AT5G64930, AT4G24290, AT5G64570,AT3G04740, AT2G39200, AT3G50930, AT5G18610, AT4G37930 |
| | EFN* density | Glyceraldehyde-3-phosphate metabolic process | 3.2 | 0.2 | 0.09 | 0.40 | AT1G71100, AT3G55440, AT4G13830, AT2G26930 |
| Leaf galling insects | Galling insects (P/A)[†] | Cellular respiration | 4.9 | 0.5 | 0.05 | 0.32 | AT1G08480, AT2G20420, AT5G51060, AT5G03860, AT5G09600 |
| | | Aerobic respiration | 3.9 | 0.3 | 0.10 | 0.60 | AT1G08480, AT2G20420, AT5G51060, AT5G03860 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | *Ecteodemia populella* (P/A)† | Anthocyanin-containing compound biosynthetic process | 3.4 | 0.1 | 0.01 | 0.02 | AT4G01900, AT3G28430, AT5G13930, AT4G14090 |
| | | Tricarboxylic acid cycle | 4.2 | 0.2 | 0.07 | 0.10 | AT1G08480, AT2G20420, AT5G03860, AT5G62575 |
| | | Citrate metabolic process | 3.4 | 0.2 | 0.07 | 0.10 | AT1G08480, AT2G20420, AT5G03860, AT5G62575 |
| | | Anthocyanin-containing compound metabolic process | 3.4 | 0.2 | 0.07 | 0.10 | AT4G01900, AT3G28430, AT5G13930, AT4G14090 |
| | | Tricarboxylic acid metabolic process | 3.4 | 0.3 | 0.09 | 0.10 | AT1G08480, AT2G20420, AT5G03860, AT5G62575 |
| | | Flavonoid biosynthetic process | 4.2 | 0.4 | 0.04 | 0.08 | AT4G01900, AT5G48930, AT3G28430, AT5G13930, AT4G14090 |
| | *Harmandia* (P/A)† | Cell wall biogenesis | 6.2 | 1.1 | 0.08 | 0.20 | AT4G03210, AT3G26370, AT1G19360, AT2G33460, AT3G08900, AT5G65270, AT3G02230 |
| Leaf rolling insects | Tortricidae | Response to mechanical stimulus | 2.4 | 0.1 | 0.06 | 0.14 | AT1G43700, AT5G61210, AT4G35920 |
| | Tenthredinidae (P/A)† | Intracellular sequestering of iron ion | 1.9 | 0.0 | 0.08 | 0.26 | AT2G01770, AT3G11050 |
| | | Sequestering of iron ion | 1.9 | 0.0 | 0.08 | 0.26 | AT2G01770, AT3G11050 |
| Free feeding insects | Free feeders (P/A)† | Peroxisome organization | 3.4 | 0.2 | 0.03 | 0.08 | AT1G63900, AT3G19720, AT1G48635, AT3G18160 |
| Ants | Formicidae | Gamma-aminobutyric acid transport | 2.0 | 0.0 | 0.03 | 0.04 | AT2G01170, AT1G08230 |
| | Formicidae (P/A)† | | 2.0 | 0.0 | 0.02 | 0.18 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Multivariate insect traits | NMDS‡ of insect species | Cellular response to acid chemical | 10.2 | 2.3 | 0.01 | 0.00 | AT4G04720, AT5G11260, AT5G66730, AT5G64930, AT1G27320, AT2G40830, AT1G78380, AT2G18470, AT3G50530, AT3G56850, AT2G18790, AT4G34220 |
| | NMDS‡ of insect species (P/A)† | Polyol catabolic process | 2.3 | 0.1 | 0.04 | 0.40 | AT2G43900, AT2G21170, AT4G18010 |
| | NMDS‡ of insect families | Glycosyl compound metabolism process | 5.8 | 0.8 | 0.05 | 0.10 | AT4G24340, AT2G22840, AT5G28050, AT1G27320, AT1G03110, AT4G21760 |
| | NMDS‡ of insect guilds** | Gamma-aminobutyric acid transport | 1.9 | 0.0 | 0.03 | 0.34 | AT2G01170, AT1G08230 |
| | NMDS‡ of insect guilds** (P/A)† | Gamma-aminobutyric acid transport | 1.8 | 0.0 | 0.03 | 0.22 | AT2G01170, AT1G08230 |
| | | Hormone metabolic process | 7.1 | 1.3 | 0.04 | 0.22 | AT5G06300, AT1G04610, AT5G57740, AT3G30180, AT2G28305, AT1G17420, AT1G44350, AT4G02680 |
| | | Hormone biosynthetic process | 6.2 | 1.0 | 0.05 | 0.26 | AT1G17420, AT5G06300, AT1G04610, AT4G02680, AT5G57740, AT3G30180, AT2G28305 |
| | | Regulation of hormone levels | 8.0 | 1.8 | 0.08 | 0.32 | AT5G06300, AT1G04610, AT5G57740, AT3G30180, AT2G28305, AT1G17420, AT1G44350, AT4G02680, AT5G56750 |
| | Insect guilds** (P/A)† | Xyloglucan biosynthetic process | 2.2 | 0.1 | 0.02 | 0.02 | AT5G04885, AT1G14100, AT2G03220 |

* EFN = extra floral nectary

** Insect guilds = leaf gallers, miners, rollers, free feeders, aphids, ants

† P/A = presence/absence

‡ NMDS = nonmetric multidimensional scaling

§ FDR = false discovery rate

**Table 3.** Summary of tree trait covariates that eliminate or reduce the number of significant SNPs associated with particular insect traits for the WisAsp aspen (*Populus tremuloides*) genetic mapping population (N = 328 genets for insect traits). Covariates that reduce SNP associations reveal tree traits that are important in shaping the particular insect phenotype, and are indicated by an “X” below. Genome-wide association models were analyzed in PLINK without a kinship matrix and standardized tree traits (each tree trait was analyzed in separate models).

| Insect trait with significant SNP associations | Tree size/growth | | | | | | | Leaf morphology | | | Bud phenology | | | Phytochemistry | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Spring volume | Spring basal area | Average basal area | Average volume | Absolute growth | Relative growth | Basal area increment | Specific leaf area | Individual leaf area | EFN* density | Timing of bud break | Timing of bud set | Growing season length | Condensed tannins | Salicortin | Tremulacin | Phenolic glycosides** | Total defense chemistry*** | Carbon:nitrogen | Nitrogen |
| *Ecteodemia populella* (P/A)† | | | | | | X | | | | | | | | | | | | | | |
| BlotchMine (P/A)† | | | | | | | | | X | X | | | | X | X | | X | X | X | |
| *Phyllonorycter tremuloidiella* (P/A)† | X | X | X | | | X | X | | X | | X | | | | X | X | X | | | |
| *Clostera albosigma* (P/A)† | | | | | X | X | X | | X | | X | | | | X | X | X | | | |
| *Choristoneura rosaceana* | | X | X | X | | | | | | | X | X | | X | X | X | X | | | |
| Cecidomyiidae | | | | | X | | | | X | X | | | | | X | | X | | | |
| Cecidomyiidae (P/A)† | | | | | | | | | X | X | X | | | X | X | X | X | | | |
| Tortricidae | | X | X | X | | | X | | X | | X | | | X | X | X | X | | | |
| *Lasius neoniger* | | | | | | | | | X | | | | | X | X | X | X | | | |
| *Lasius neoniger* (P/A)† | | | | | | | | | X | | | | | | X | X | X | | | |

* EFN = extra floral nectary

** Phenolic glycosides = combined levels of salicortin and tremulacin

*** Total defense chemistry = combined levels of salicortin, tremulacin, and condensed tannins

† P/A = presence/absence

**Figure 1.** (A) Map of Wisconsin that displays the origin of the WisAsp aspen genets (white points, shown with 30% transparency) and the WisAsp common garden (red point). (B) Kinship matrix for the 445 genets (white represents unrelated genets, black represents 100% relatedness, *i.e.,* comparing a genet to itself, and gray represents intermediate relatedness between genets). (C) Zoomed in section of the kinship matrix, showing a few of the sibling pairs (gray squares).

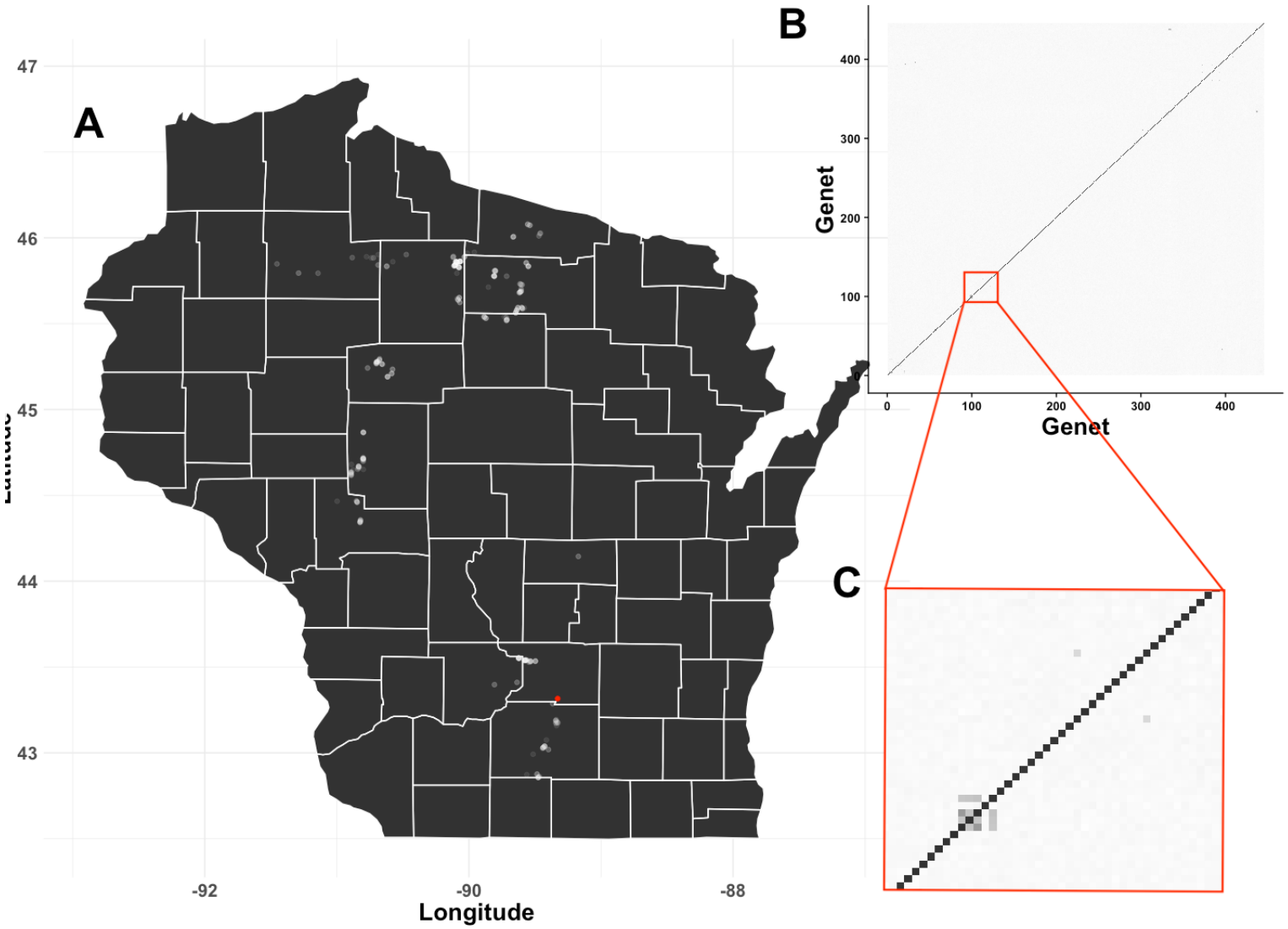
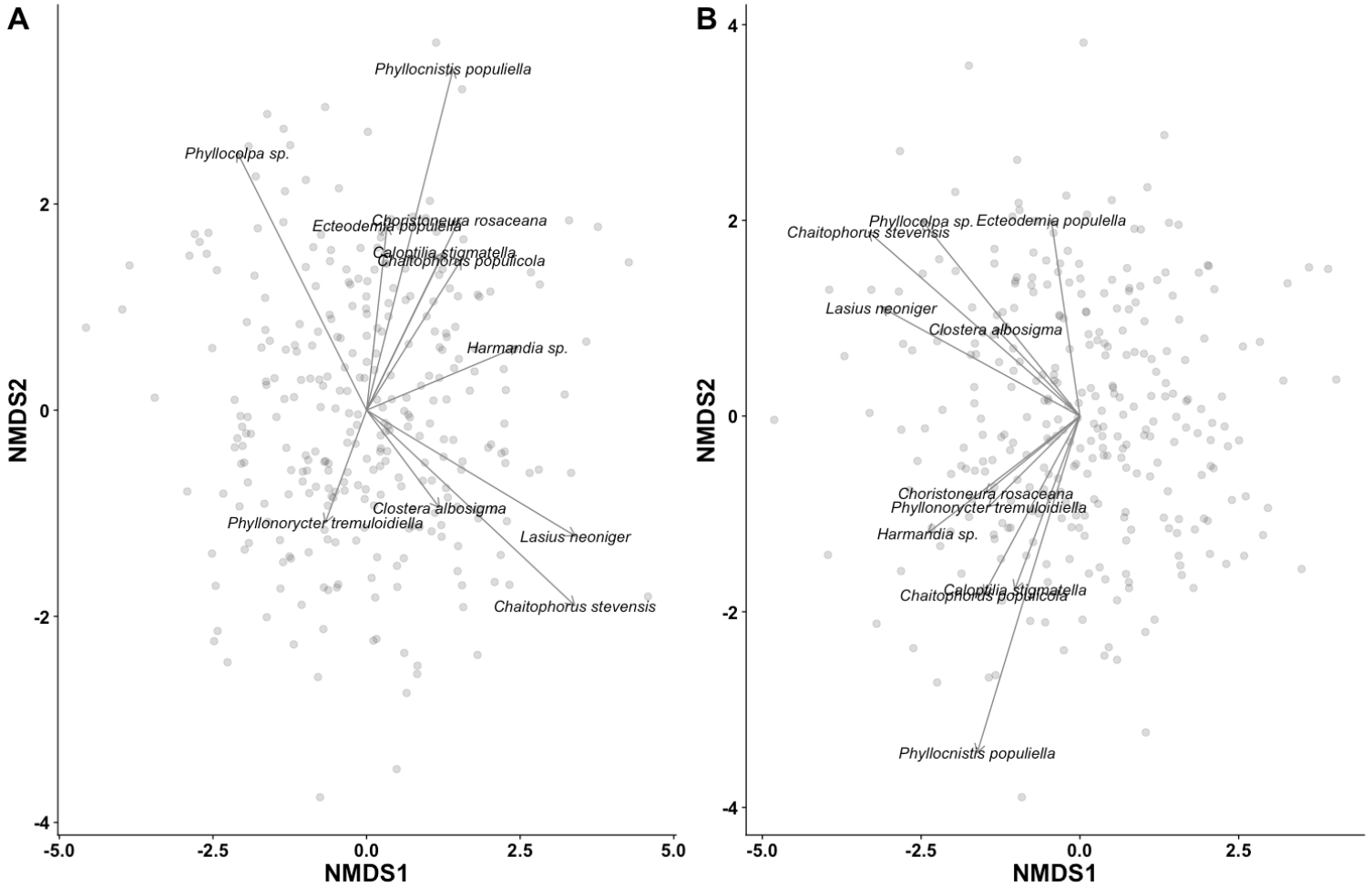

A
B
C
47
46
45
44
43
-92
-90
-88
Longitude
Genet
400
300
200
100
0
100
200
300
400
Genet

**Figure 2.** Nonmetric multidimensional scaling (NMDS) ordination for both the (A) abundance and (B) incidence of common insect species surveyed on aspen in the WisAsp common garden (2014-5). Each point represents an insect community on an aspen genet. Each vector shows the insect species-level changes in the community (*i.e.,* along a given vector, the particular insect species is either more [A] abundant or more [B] present within the insect community). (C) Summary of significant single nucleotide polymorphism (SNP) associations (false discovery rate [FDR] q-value < 0.10) for multivariate insect phenotypes for the WisAsp *Populus tremuloides* genetic mapping population (N = 328 genets). Genome-wide association models were analyzed in PLINK without a kinship matrix. Bolded alleles are the minor allele.




**C**

| Trait | *P. tremula* (v1.1) | SNP position | Alleles | MAF | P-value | FDR q-value | Homologous gene: *P. tremuloides* (v1.1) | Homologous gene: *P. trichocarpa* (v3.0) | Homologous gene: *A. thaliana* | Gene annotation | NMDS1 | NDMS2 | NMDS3 | NMDS4 | NMDS5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NMDS‡ of insect species | Potra001060g09097 | 68122 | C T | 0.136 | 3.41E-07 | 0.029 | Potrs000733g01118 | Potri.003G050500 | AT1G79800 | Nodulin-like protein | 0.801 | -0.174 | 0.060 | -0.561 | 0.120 |
| | | 68195 | C T | 0.136 | 3.15E-07 | 0.029 | | | | | 0.821 | -0.180 | 0.095 | -0.530 | 0.093 |
| NMDS‡ of insect species (P/A)† | Potra001060g09097 | 68122 | C T | 0.136 | 5.24E-08 | 0.007 | Potrs000733g01118 | Potri.003G050500 | AT1G79800 | Nodulin-like protein | -0.696 | 0.195 | -0.518 | -0.162 | 0.425 |
| | | 68195 | C T | 0.136 | 7.85E-08 | 0.007 | | | | | -0.718 | 0.194 | -0.497 | -0.168 | 0.410 |

† P/A = presence/absence

‡ NMDS = nonmetric multidimensional scaling

**STable 1.** Partial least squares model fit for predicting chemical constituents with near infrared spectroscopy (NIRS).

| Constituent | N | Mean | SD | Est. Min | Est. Max | SEC | $R^2$ | SECV | RPD |
|---|---|---|---|---|---|---|---|---|---|
| Condensed tannins | 336 | 15.32 | 6.84 | 0 | 35.85 | 1.18 | 0.970 | 1.24 | 5.50 |
| Tremulacin | 292 | 4.02 | 2.45 | 0 | 11.38 | 0.42 | 0.971 | 0.48 | 5.05 |
| Salicortin | 296 | 5.34 | 2.36 | 0 | 12.41 | 0.51 | 0.954 | 0.57 | 4.11 |
| Nitrogen | 165 | 2.14 | 0.28 | 1.3 | 2.99 | 0.04 | 0.979 | 0.05 | 5.94 |

N = sample size used to make prediction model
SD = standard deviation of predicted values
Est. Min = estimated minimum predicted value
Est. Max = estimated maximum predicted value
SEC = standard error of calibration
$R^2$ = coefficient of determination
SECV = standard error of cross validation
RPD = relative percent difference

**STable 2.** Single nucleotide polymorphism (SNP) filtering pipeline for the WisAsp genetic mapping population of trembling aspen (*Populus tremuloides*).

| SNP filtering program | SNP/Sample filters | Number of SNPs retained | Number of removed SNPs | Percent of removed SNPs | Number of Transitions (Ts) | Number of Transversions (Tv) | Ts/Tv | Number of singletons |
|---|---|---|---|---|---|---|---|---|
| **SNP – calling** | **1.** remove SNPs that are outside the extended probe regions<br>**2.** remove low quality SNP calls | 1,958,501 | NA | NA | 730,699 | 623,410 | 1.17 | 149,564 |
| **BCFtools** | **1.** quality by depth (QD 2)<br>**2.** root mean square of the mapping quality of the reads across all samples (MQ 50)<br>**3.** Mann-Whitney rank sum test for mapping qualities (MQRankSum -8)<br>**4.** Mann-Whitney rank sum test for the distance from the end of the reads for reads with alternative alleles (ReadPosRankSum -8)<br>**5.** exclude high depth outliers (INFO/DP 5000) | 1,609,672 | 348,829 | 18% | 612,014 | 512,429 | 1.19 | 152,771 |
| **VCFtools** | **1.** keep SNPs with 2 alleles (--min-allele 2 max-allele 2) | 883,708 | 725,964 | 45% | 485,037 | 398,671 | 1.22 | 230,040 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **2.** keep SNPs with mean depth of 6-90 (--min-meanDP 6 --max-meanDP 90)<br>**3.** remove indels and 5bp around indels (--remove-indels)<br>**4.** remove SNPs with more than 30% missing data (--max-missing 0.7)<br>**5.** remove SNPs with a genotype quality less than 30 (--minGQ 30)<br>**6.** remove SNPs with Hardy-Weinberg p-values <0.00001 (--HWE 0.00001)<br>**7.** remove samples with >20% missing data (--remove) | | | | | | | |
| **VCFtools** | merge SNP data from probe samples (N=434) and whole genome samples (N=11, vcf-merge) | 1,554,838 | NA | NA | 739,879 | 611,315 | 1.21 | 506,303 |
| **VCFtools** | **1.** remove SNPs with more than 30% missing data (--max-missing 0.7)<br>**2.** keep SNPs with 2 alleles (--min-allele 2 max-allele 2)<br>**3.** remove indels and 5bp around indels (--remove-indels) | 816,881 | 66,827 | 8% | 448,895 | 367,986 | 1.22 | 226,208 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **4.** remove SNPs with a genotype quality less than 30 (--minGQ 30)<br>**5.** remove SNPs with a minor allele frequency <0.00001 (--maf 0.00001)<br>**6.** remove SNPs with Hardy-Weinberg p-values <0.00001 (--HWE 0.00001) | | | | | | | |
| **VCFtools** | remove SNPs with a minor allele frequency <0.05 (--maf 0.05) | 173,513 | 643,368 | 79% | 99,359 | 74,154 | 1.34 | 0 |
| **LinkImpute** | impute missing genotypes | 173,513 | 0 | 0% | 99,354 | 74,152 | 1.34 | 0 |
| **PLINK** | prune by linkage disequilibrium (–indep-pairwise 50 10 0.2) | 139,338 | 34,175 | 19.70% | 80,353 | 58,985 | 1.36 | 0 |

**STable 3.** Summary of all genome-wide association and gene set enrichment analyses for the WisAsp aspen (*Populus tremuloides*) genetic mapping population (N = 445 genets for tree traits, N = 328 genets for insect traits).

| Trait category | Trait | Number of SNPs (FDR q-value < 0.10) | Enriched GO terms? (Yes/No) |
|---|---|---|---|
| Tree growth/size | Spring volume | 0 | Y |
| | Spring basal area | 0 | Y |
| | Average basal area | 0 | Y |
| | Average volume | 0 | Y |
| | Absolute growth | 0 | Y |
| | Relative growth | 0 | N |
| | Basal area increment | 0 | Y |
| Leaf morphology | Specific leaf area | 0 | Y |
| | Individual leaf area | 0 | N |
| | EFN density | 0 | Y |
| Foliar phenology | Bud break date | 0 | N |
| | Bud set date | 0 | N |
| | Growing season length | 0 | N |
| Phytochemistry | Condensed tannins | 0 | N |
| | Phenolic glycosides | 2 | N |
| | Tremulacin | 2 | N |
| | Salicortin | 0 | N |
| | Total defense chemistry | 1 | N |
| | C:N | 0 | N |
| | Nitrogen | 0 | N |
| Multivariate tree traits | Relative and absolute growth, specific leaf area, individual leaf area, EFN density, growing season length, and levels of condensed tannins, phenolic glycosides, and nitrogen | 0 | N |
| Leaf-rolling insects | *Phyllocolpa* sp. 1 | 0 | N |
| | *Phyllocolpa* sp. 1 (P/A) | 0 | N |
| | *Clostera albosigma* | 1 | N |
| | *Clostera albosigma* (P/A) | 1 | N |
| | *Choristoneura rosaceana* | 2 | N |
| | *Choristoneura rosaceana* (P/A) | 0 | N |
| Leaf-mining insects | *Phyllonorycter tremuloidiella* | 0 | N |
| | *Phyllonorycter tremuloidiella* (P/A) | 3 | N |
| | *Caloptilia stigmatella* | 0 | N |
| | *Caloptilia stigmatella* (P/A) | 0 | N |
| | *Phyllocnistis populiella* | 0 | N |

| | | | |
|---|---|---|---|
| | *Phyllocnistis populiella* (P/A) | 0 | N |
| Leaf-galling insects | *Harmandia* sp. 1 | 0 | N |
| | *Harmandia* sp. 1 (P/A) | 0 | Y |
| | *Ecteodemia populella* | 0 | N |
| | *Ecteodemia populella* (P/A) | 5 | Y |
| Free-feeding insects | *Chaitophorus populicola* | 0 | N |
| | *Chaitophorus populicola* (P/A) | 0 | N |
| | *Chaitophorus stevensis* | 0 | N |
| | *Chaitophorus stevensis* (P/A) | 0 | N |
| Ants | *Lasius neoniger* | 12 | N |
| | *Lasius neoniger* (P/A) | 12 | N |
| Insect families | Cecidomyiidae | 1 | N |
| | Cecidomyiidae (P/A) | 1 | N |
| | Tenthredinidae | 0 | N |
| | Tenthredinidae (P/A) | 0 | Y |
| | Gracilliaridae | 0 | N |
| | Agromyzidae (P/A) | 0 | N |
| | Tortricidae | 2 | Y |
| | Tortricidae (P/A) | 0 | N |
| | Aphididae (P/A) | 0 | N |
| | Formicidae | 0 | Y |
| | Formicidae (P/A) | 0 | Y |
| | Chrysomelidae | 0 | N |
| | Chrysomelidae (P/A) | 0 | N |
| | Pentatomidae | 0 | N |
| Insect guilds | Leaf-miners | 0 | N |
| | Leaf-miners (P/A) | 0 | N |
| | Leaf-rollers | 0 | N |
| | Leaf-rollers (P/A) | 0 | N |
| | Leaf-gallers | 0 | N |
| | Leaf-gallers (P/A) | 0 | Y |
| | Free feeders | 0 | N |
| | Free feeders (P/A) | 0 | Y |
| Insect community metrics | Total insect abundance | 0 | N |
| | Species richness | 0 | N |
| | Shannon index | 0 | N |

| | | | |
|---|---|---|---|
| Multivariate insect traits | NMDS of the abundance of common species | 2 | Y |
| | NMDS of the incidence of common species | 2 | Y |
| | NMDS of the abundance of common families | 0 | Y |
| | NMDS of the incidence of common families | 0 | N |
| | NMDS of the abundance of common guilds | 0 | Y |
| | NMDS of the incidence of common guilds | 0 | Y |
| | Abundance of guilds | 0 | N |
| | Incidence of guilds | 0 | Y |
| | Combined community metrics | 0 | N |

GO = gene ontology
EFN = extra floral nectary
C:N = foliar carbon:nitrogen ratio
P/A = presence/absence
Common = insect that was found on >5% of the surveyed trees
NMDS = nonmetric multidimensional scaling axes (N = 5 axes for species- and family-level analyses, and N = 3 for guild-level models)

**STable 4.** Summary of significant single nucleotide polymorphism (SNP) associations (false discovery rate [FDR] q-value < 0.10) for tree traits and insect phenotypes for the WisAsp *Populus tremuloides* genetic mapping population (N = 445 genets for tree traits, N = 328 genets for insect traits). Genome-wide association models were analyzed using GEMMA with a centered kinship matrix. Bolded alleles are the minor allele.

| Trait | *P. tremula* (v1.1) | SNP position | Alleles | | MAF‡ | P-value | FDR q-value | Beta (SE)§ | Homologous gene: *P. tremuloides* (v1.1) | Homologous gene: *P. trichocarpa* (v3.0) | Homologous gene: *A. thaliana* | Gene annotation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tremulacin | Potra003979g23949 | 37860 | **A** | T | 0.054 | 4.49E-07 | 0.038 | 0.779 (0.151) | Potrs002611g30592 | Potri.004G141000 | AT3G19260 | ASC1-like protein |
| | | 38233 | **G** | C | 0.054 | 4.49E-07 | 0.038 | 0.779 (0.151) | | | | |
| Phenolic glycosides* | Potra003979g23949 | 37860 | **A** | T | 0.054 | 3.84E-07 | 0.033 | 0.787 (0.152) | Potrs002611g30592 | Potri.004G141000 | AT3G19260 | ASC1-like protein |
| | | 38233 | **G** | C | 0.054 | 3.84E-07 | 0.033 | 0.787 (0.152) | | | | |
| Condensed tannins | Potra002059g16058 | 14094 | **C** | T | 0.056 | 5.64E-07 | 0.097 | -0.717 (0.144) | Potrs003097g04940 | Potri.001G215700 | AT1G08420 | Serine/threonine-protein |
| *Ecteodemia populella* (P/A)† | Potra001062g09110 | 18542 | **C** | T | 0.059 | 3.40E-07 | 0.047 | -0.886 (0.171) | Potrs042256g26529 | Potri.018G046100 | AT2G25770 | Abscisic acid receptor |
| | Potra001062g09111 | 28996 | **T** | A | 0.062 | 1.12E-06 | 0.047 | -0.826 (0.167) | Potrs007988g11232 | Potri.018G046200 | AT1G15215 | Protein SAWADEE HOMEODOMAIN HOMOLOG |
| | | 29108 | **A** | C | 0.062 | 1.12E-06 | 0.047 | -0.826 (0.167) | | | | |
| | | 29109 | **T** | A | 0.062 | 1.12E-06 | 0.047 | -0.826 (0.167) | | | | |
| | | 29512 | **T** | C | 0.054 | 1.36E-06 | 0.047 | -0.875 (0.178) | | | | |
| *Phyllonorycter tremuloidiella* (P/A)† | Potra000892g07232 | 177159 | **C** | T | 0.249 | 1.15E-06 | 0.066 | -0.464 (0.094) | Potrs009143g14209 | Potri.007G124400 | AT5G01430 | Vesicle transport protein |
| | | 177288 | **C** | T | 0.249 | 1.15E-06 | 0.066 | -0.464 (0.094) | | | | |
| | | 177289 | **A** | G | 0.249 | 1.15E-06 | 0.066 | -0.464 (0.094) | | | | |
| *Choristoneura rosaceana* | Potra000544g03858 | 259777 | **C** | T | 0.436 | 4.53E-07 | 0.043 | -0.460 (0.092) | Potrs019807g22433 | Potri.006G237800 | AT5G11630 | Uncharacterized protein LOC105122277 |
| | | 262984 | **A** | T | 0.435 | 5.02E-07 | 0.043 | -0.460 (0.093) | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tortricidae | Potra000544g03858 | 259777 | **C** | T | 0.436 | 4.45E-07 | 0.042 | -0.460 (0.092) | Potrs019807g22433 | Potri.006G237800 | AT5G11630 | Uncharacterized protein LOC105122277 |
| | Potra000544g03859 | 262984 | **A** | T | 0.435 | 4.90E-07 | 0.042 | -0.460 (0.093) | Potrs042389g26631 | Potri.002G045500 | AT5G43680 | Sec-independent protein translocase protein TatB |
| *Lasius neoniger* | Potra003266g21171 | 16319 | **A** | T | 0.305 | 1.03E-06 | 0.026 | 0.392 (0.080) | Potrs017980g20216 | Potri.001G287600 | AT3G24730 | Thioredoxin-like protein |
| | | 16337 | **A** | G | 0.305 | 1.03E-06 | 0.026 | 0.392 (0.080) | | | | |
| | | 16338 | **G** | A | 0.305 | 1.42E-06 | 0.030 | 0.384 (0.080) | | | | |
| | | 16381 | **A** | G | 0.303 | 8.94E-07 | 0.026 | 0.397 (0.081) | | | | |
| | | 16473 | **T** | C | 0.303 | 8.94E-07 | 0.026 | 0.397 (0.081) | | | | |
| | | 16480 | **G** | A | 0.303 | 8.94E-07 | 0.026 | 0.397 (0.081) | | | | |
| | | 16548 | **G** | A | 0.300 | 7.39E-07 | 0.026 | 0.405 (0.081) | | | | |
| | | 16549 | **G** | A | 0.300 | 7.39E-07 | 0.026 | 0.405 (0.081) | | | | |
| | Potra002557g19270 | 32237 | **G** | A | 0.119 | 3.26E-06 | 0.047 | 0.540 (0.115) | Potrs004787g06948 | Potri.018G040200 | AT4G32770 | Tocopherol cyclase, chloroplastic |
| | | 32326 | **T** | C | 0.119 | 3.26E-06 | 0.047 | 0.540 (0.115) | | | | |
| | | 32327 | **C** | A | 0.119 | 3.26E-06 | 0.047 | 0.540 (0.115) | | | | |
| | Potra003286g21239 | 28246 | **T** | C | 0.071 | 1.56E-06 | 0.030 | -0.771 (0.154) | Potrs009219g14285 | Potri.019G126800 | AT2G40435 | Uncharacterized protein LOC105123201 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lasius neoniger* (P/A)[†] | Potra003266g21171 | 16319 | **A** | T | 0.305 | 6.92E-07 | 0.017 | -0.398 (0.080) | Potrs017980g20216 | Potri.001G287600 | AT3G24730 | Thioredoxin-like protein |
| | | 16337 | **A** | G | 0.305 | 6.92E-07 | 0.017 | -0.398 (0.080) | | | | |
| | | 16338 | **G** | A | 0.305 | 9.63E-07 | 0.021 | -0.390 (0.079) | | | | |
| | | 16381 | **A** | G | 0.303 | 6.28E-07 | 0.017 | -0.402 (0.080) | | | | |
| | | 16473 | **T** | C | 0.303 | 6.28E-07 | 0.017 | -0.402 (0.080) | | | | |
| | | 16480 | **G** | A | 0.303 | 6.28E-07 | 0.017 | -0.402 (0.080) | | | | |
| | | 16548 | **G** | A | 0.300 | 5.11E-07 | 0.017 | -0.411 (0.081) | | | | |
| | | 16549 | **G** | A | 0.300 | 5.11E-07 | 0.017 | -0.411 (0.081) | | | | |
| | | 30593 | **C** | T | 0.119 | 7.33E-06 | 0.097 | -0.523 (0.116) | | | | |
| | Potra002557g19270 | 32237 | **G** | A | 0.119 | 3.00E-06 | 0.043 | -0.542 (0.115) | Potrs004787g06948 | Potri.018G040200 | AT4G32770 | Tocopherol cyclase, chloroplastic |
| | | 32326 | **T** | C | 0.119 | 3.00E-06 | 0.043 | -0.542 (0.115) | | | | |
| | | 32327 | **C** | A | 0.119 | 3.00E-06 | 0.043 | -0.542 (0.115) | | | | |
| | Potra003286g21239 | 28246 | **T** | C | 0.071 | 1.36E-06 | 0.026 | 0.776 (0.154) | Potrs009219g14285 | Potri.019G126800 | AT2G40435 | Uncharacterized protein LOC105123201 |
| *Chaitophorus stevensis* (P/A)[†] | Potra003737g22637 | 26211 | **G** | A | 0.498 | 4.81E-07 | 0.081 | 0.398 (0.078) | Potrs003384g05131 | Potri.002G075300 | AT3G26540 | Repeat-containing protein |

* Phenolic glycosides = combined levels of salicortin and tremulacin
[†] P/A = presence/absence
[‡] MAF = minor allele frequency
[§] SE = standard error

**STable 5.** Summary of probe coverage using *P. trichocarpa* homologs with the Knetminer (Hassani-Pak 2017) search engine. I conducted five searches using different sets of search terms to find the list of *Populus* genes that have been shown to underlie or interact with these traits. I then compared my gene list to the returned gene list to assess my probe coverage.

| **Knetminer search terms (using *P. trichocarpa* homologs)** | **Number of returned genes** | **Number of genes not covered by probes** | **Number of genes covered by probes** | **Percent of genes not covered by probes** | **Percent of genes covered by probes** |
|---|---|---|---|---|---|
| "phenylpropanoid pathway" | 11810 | 4456 | 7354 | 38% | 62% |
| "bud formation" OR "leaf phenology" OR "flowering time" | 3347 | 987 | 2360 | 29% | 71% |
| "biomass" OR "biomass yield" OR "tree size" | 2850 | 832 | 2018 | 29% | 71% |
| "SLA" OR "leaf area" | 383 | 96 | 287 | 25% | 75% |
| "insect" OR "herbivory" OR "insect damage resistance" | 3550 | 1648 | 1902 | 46% | 54% |

**SFigure 1.** Bud break scale: (1) dormant bud, (2) green and enlarged bud, (3) broken bud, (4) leaves flushed out but rolled, and (5) leaves flushed out and completely unrolled.


1
2
3
4
5

**SFigure 2.** WisAsp common garden in 2014. Photo by Mary Jamieson.

## Thesis conclusions

Intraspecific variation shapes community and ecosystem patterns. This effect is most prominent in foundation tree species, which play a critical role in regulating species interactions, the composition of dependent communities (*e.g.,* arthropods, pathogens, endophytes, etc.), and ecosystem dynamics (*e.g.,* nutrient cycling). Previous work has shown that the composition of dependent communities is a heritable trait of foundation tree species, and that tree traits act as the mechanistic link between tree genetics and these dependent communities. However, this work has generally focused on the tree genet-level and has surveyed a limited set of tree traits. Thus, the underlying tree genes that structure dependent communities and the traits that link tree genes to community composition have remained largely unresolved. In addition, this work is often limited to common garden designs that do not incorporate environmental variation. Therefore, the effects of tree genetics, the environment, and their interaction (GxE) in shaping ecologically-relevant tree traits and extended phenotypes (*e.g.,* insect herbivore performance) required further synthesis. My doctoral research sought to address these gaps to improve our understanding of both the causes and effects of intraspecific variation in foundation tree species. My key findings are summarized below.

1. **Chapter 1: Salicaceae defense phytochemistry and insect performance were primarily genetically-determined, while plant growth and foliar nitrogen were largely environmentally-determined.** Both salicinoids and condensed tannins were highly heritable ($H^2 > 0.50$), and while this result was expected for salicinoids, it contradicted some previous findings for variation in

condensed tannins. Condensed tannins have exhibited great plasticity in particular environments (varying competition and light treatments), which were rare in my meta-analysis.

2. **Chapter 1: Phenotypic plasticity varied across Salicaceae traits and environmental conditions.** Plasticity was highest for plant growth, foliar nitrogen, and insect herbivore performance for plants grown across different sites, and soil nutrient, water, and carbon dioxide levels. Conversely, plasticity was lowest for chemical defense traits and was low for all traits in contrasting ozone and defoliation environments.

3. **Chapter 2:** I**nsect communities in the aspen canopy overall had low ($H^2$ <0.16) and variable heritability estimates.** Insects that interact more closely with their aspen host, such as leaf-galling insects, had the highest heritability, whereas more mobile insects that are less intimately associated with their aspen host, such as free-feeding insects had the lowest heritability.

4. **Chapter 2: Few genotype- and phenotype-based species interactions were identified among canopy insects.** Of these significant relationships, both genotype- and phenotype-based species interactions were relatively strong between key insect species (aphids and ants) and other community members (*e.g.,* free feeders). This finding is consistent with the Interacting Foundation

Species Hypothesis in that relationships among key species (aspen, aphids, ants) shape the larger community (canopy insects).

5. **Chapter 2: Several tree traits influenced insect community metrics and the presence/absence of common insect species and functional groups.** Most notably, tree size had a dramatic effect, with denser and more diverse insect communities found on larger trees, while foliar bud phenology was the most important trait for differentially affecting insect species and functional groups. In particular, leaf-modifying insect species were sensitive to the timing of bud break and set. Contrary to my expectations, levels of salicinoids did not appear to structure the composition of canopy insect communities. This result may have stemmed from the prevalence of specialist insects within the communities and/or the timing of leaf collection for chemical analyses (late June) in relation to insect surveys (mid July to early August).

6. **Chapter 3: My findings revealed new gene associations for variation in defense compounds, including salicinoids, which helps to shed light on their phytochemical pathway**. An ASC1-like protein (Cyp1 Absence of growth Suppressor, *Potra003979g23949*) was associated with both levels of salicinoids and tremulacin in particular, whereas a ribosomal protein (*Potra007960g26067*) was associated with total chemical defense (combined condensed tannin and salicinoid levels). These genes are active in gene expression and respond to plant hormones.

7. **Chapter 3: My findings also revealed 11 new gene associations for variation in the abundance and incidence of leaf modifying insects and ants, and variation in insect community composition.** I found associated genes and gene functions that reflect the series of plant-insect events that follow insect herbivory (damage-induced ion imbalances, differing membrane potentials, calcium signaling, oxidative stress, altered kinase and hormone activity, changes in gene expression). In addition, I found genetic associations for galling insects that correspond directly with results from a gene expression study in grape galls (increased cell wall biogenesis and anthocyanin production). My findings also revealed an apoptosis-inducing gene, *Potra002833g20082*, that may confer resistance to gall formation. Most notably, I identified an early nodulin-like (ENODL) transmembrane protein (*Potra001060g09097*) that was correlated with insect community composition. Allelic variation in this gene resulted in differences in insect species diversity and abundance of key foundation species: aphids and ants.

8. **Chapter 3: Gene-insect associations were shaped (directly or indirectly) by ecologically-relevant tree traits.** Tree size, individual leaf area, extra-floral nectary density, bud phenology, condensed tannins and salicinoids influenced the gene-associated variation in leaf-rolling, leaf-mining, leaf-galling, and petiole-galling insects and ants.

**Recommendations for future research**

My meta-analysis revealed several underrepresented factors in the Salicaceae literature that need further investigation. In addition, my findings in the mechanisms that shape canopy insect communities highlight several areas for future work.

These areas include:

1. Further research should study controlled manipulations of the effects of biotic environments (competition, defoliation, disease, etc.) and light and water treatments on Salicaceae populations. In addition, research on Salicaceae-insect interactions is needed that surveys various leaf-modifying insect species and specialist feeders. To date, most Salicaceae studies that assess insect performance focus on only two generalist lepidopterans: gypsy moths (*Lymantria dispar dispar*) and forest tent caterpillars (*Malacosoma disstria*).

2. Future meta-analyses could partition plant phenotypic variation to determine whether these patterns hold across different plant taxa (*e.g.,* temperate vs. tropical species, crops vs. natural populations, taxa with different phytochemical defense compounds).

3. Manipulative experiments are needed to assess how canopy insect communities are shaped by the plant's genetic variation, environmental context, and the interaction between plant genotype and environment. In addition, this work could

determine whether plant genes that underlie canopy insect communities vary with environmental factors.

4. My thesis studied a relatively young (4-5 years old) population of aspen. Future work could assess how ontogenetic trajectories may change ecologically-relevant tree traits, canopy insect communities, and underlying genes.

5. Gene knockout/modification studies could follow-up significant gene associations to determine the effect of these particular genes on aspen traits (defense compounds) and canopy insect communities.

6. Future community genetics research should continue to focus on identifying *mechanisms* underlying the effects of plant intraspecific variation on associated communities, rather than simply quantifying patterns.