

Evaluating the Effects of Aspen Genetics on Insect Communities - Draft

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Introduction

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

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

To address phenotypic mechanisms of genotype-based community effects, we propose to evaluate the combined effects that plant genetics and traits have in shaping associated insect herbivore communities, using a *Populus* experimental system. To this end, we will use a model-based approach to test the collective effects of (1) *Populus tremuloides* (aspen) genotype at various SNPs, (2) various aspen traits including size and defense traits, and (3) various environmental factors including weather and time on the abundance of 18 common insect species.

Background

Community genetics: state of the field

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

mediated by genes. Phenotypic traits, then, are the mechanisms of community genetic effects (Hersch-Green et al 2011; Crutsinger 2016).

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

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

Populus tremuloides as a study system

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

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

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

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

Methods

Data Collection

Experimental Design

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

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

Data collection

Data were collected on tree genetics, traits, and associated insect communities. Insect community data were collected by conducting visual surveys of insect herbivores on the lower third of each tree’s canopy. Trees were surveyed for a predetermined duration, with 3-minute minimum and 10-minute maximum caps, based on relative height, to estimate insect density (as a function of time). out of 220 total species of insects, 18 were identified as common (occured on ≥ 5 trees in each survey event) and will be used in the analysis. Phytochemical analyses were conducted on leaf tissue from each ramet at each insect survey event. PGs were extracted from leaf tissue and quantified using UHPLC-mass spectrometry following methods of Abreu, modified by the Lindroth Lab (Abreu et al 2011; F Rubert-Nason et al 2017). CTs were extracted and quantified following the methods of Porter/Hagerman (Porter et al 1985; Hagerman and Butler 1989). Carbon and Nitrogen concentrations were quantified using near infrared spectroscopy (NIRS) with nitrogen analyzer calibration (Rubert-Nason et al 2013). All phytochemical extractions were done on leaves that had been vacuume-dried for 48 hours. Other tree traits quantified (volume, basal area, number of flowers, sex, leaf area, bud break timing, and extra-floral nectaries) were done so using standard methods (**references needed**). SNP genotype data were collected using standard methods (**reference needed**).

Statistical Analyses

We will use quantitative-trait genome wide association (GWA) methods to assess the effects of aspen genotype, at 114,420 SNP locations, on 18 herbivorous insect species. Best linear unbiased predictors (BLUPs) for each of 7 quantitative tree traits will be calculated for each genotype using generalized liner mixed effects models (GLMMs) using, `lme4::glmer()` in R. These BLUPs, 3 categorical tree traits, and 9 measured environmental factors will be used as covariates in the association regression analysis. Association regression will be conducted using `plink` and the `qtl` package in R (only if there are enough SNPs with relatively even homozygous:heterozygous genotype groups $\approx 50 : 50$). Other methods will also be considered, as `plink` does not allow for interaction terms in its regression analyses.

Reference

Bibliography to come later.