


Large effect quantitative trait loci for salicinoid phenolic glycosides in *Populus*: Implications for gene discovery

Scott A. Woolbright¹  | Brian J. Rehill² | Richard L. Lindroth³ | Stephen P. DiFazio⁴ | Gregory D. Martinsen⁵ | Matthew S. Zinkgraf⁶ | Gerard J. Allan⁵ | Paul Keim⁷ | Thomas G. Whitham⁵

¹Department of Biology, University of Arkansas at Little Rock, Little Rock, AR, USA

²Department of Chemistry, US Naval Academy, Annapolis, MD, USA

³Department of Entomology, University of Wisconsin-Madison, Madison, WI, USA

⁴Department of Biology, West Virginia University, Morgantown, WV, USA

⁵Environmental Genetics and Genomics Laboratory (EnGGen), Department of Biological Sciences, Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ, USA

⁶Department of Biology, Western Washington University, Bellingham, WA, USA

⁷Department of Biological Sciences, Pathogen and Microbe Institute, Northern Arizona University, Flagstaff, AZ, USA

Correspondence

Scott A. Woolbright, Department of Biology, University of Arkansas at Little Rock, 2801 South University Avenue Little Rock, AR, 72227 USA.

Email: sawoolbright@ualr.edu

Funding information

Achievement Rewards for College Scientists Foundation; National Science Foundation, Grant/Award Number: DBI-1126840, DEB-0425908 and DEB-1340852

Abstract

Genomic studies have been used to identify genes underlying many important plant secondary metabolic pathways. However, genes for salicinoid phenolic glycosides (SPGs)—ecologically important compounds with significant commercial, cultural, and medicinal applications—remain largely undescribed. We used a linkage map derived from a full-sib population of hybrid cottonwoods (*Populus* spp.) to search for quantitative trait loci (QTL) for the SPGs salicortin and HCH-salicortin. SSR markers and primer sequences were used to anchor the map to the V3.0 *P. trichocarpa* genome. We discovered 21 QTL for the two traits, including a major QTL for HCH-salicortin ($R^2 = .52$) that colocalized with a QTL for salicortin on chr12. Using the V3.0 *Populus* genome sequence, we identified 2,983 annotated genes and 1,480 genes of unknown function within our QTL intervals. We note ten candidate genes of interest, including a BAHD-type acyltransferase that has been potentially linked to *Populus* SPGs. Our results complement other recent studies in *Populus* with implications for gene discovery and the evolution of defensive chemistry in a model genus. To our knowledge, this is the first study to use a full-sib mapping population to identify QTL intervals and gene lists associated with SPGs.

KEYWORDS

community and ecosystem genetics, defensive chemistry genes, foundation species, *Populus*, quantitative trait loci mapping, salicinoid phenolic glycoside

1 | INTRODUCTION

Unlike primary metabolites that govern “fundamental life processes,” secondary metabolites often mediate interactions between plants and their biotic and abiotic environments (Neuman, Kumar, & Imani, 2009). Many plant secondary metabolites

have important ecological functions (Lindroth & St. Clair, 2013; Steppuhn et al., 2004) as well as economical (e.g., Pickett et al., 2014), medicinal (Devore, Kang, Breteler, & Grodstein, 2013), and ethnobotanical applications. To fully exploit the potential benefits of plant secondary metabolites, we need to know the genes that underlie the metabolic pathways governing their synthesis

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. *Ecology and Evolution* published by John Wiley & Sons Ltd.

and regulation (Redding-Johanson et al., 2011). Likewise, a genetic perspective of secondary metabolism is important to our understanding of how plant chemistry evolves (and coevolves) to affect the ecology and evolution of diverse organisms whose survival is directly or indirectly dependent upon the availability of suitable plant hosts (Lamit et al., 2015).

The genetic bases of metabolic pathways controlling the synthesis of many important secondary metabolites, including nicotine (Steppuhn, Gase, Krock, Halitschke, & Baldwin, 2004), various flavonoids (Saito et al., 2013), and terpenes (Redding-Johanson et al., 2011), are well characterized. However, the pathways leading to the salicinoid phenolic glycosides (SPGs) remain almost entirely undescribed (Tsai, Harding, Tschaplinski, Lindroth, & Yuan, 2006; Tsai, Kayal, & Harding, 2006; Boeckler, Gershezon, & Unsicker, 2011; but see Chedgy, Köllner, & Constabel, 2015). The lack of genetic data for SPGs is conspicuous given their potential agricultural, medicinal, and ecological applications. For example, salicortin, one of the SPGs in our study, has recently been investigated for potential application toward the treatment and prevention of obesity (Lee et al., 2013), insulin resistance (Harbilas et al., 2013), and inflammation (Kwon et al., 2014). Ecologically, salicortin and other SPGs serve a variety of functions (reviewed in Tsai, Harding, et al., 2006; Tsai, Kayal, et al., 2006) that include protection from UV damage (Turtola et al., 2005; Warren, Bassman, Fellman, Mattinson, & Eigenbrode, 2003), responses to drought (Turtola et al., 2005), and defense against herbivores (e.g., Lindroth & St. Clair, 2013; Holeski et al., 2016; Tahvanainen et al., 1991). In the model tree genus *Populus*, plant chemistry has been shown to be the key bridge between host plant genetic variation and insect community organization (Bangert et al., 2006; Bernhardson et al., 2013; Martinsen et al., 1998). For example, variation in leaf salicortin and other SPGs can alter herbivory by important arthropod pests, such as gypsy moths (Couture et al., 2016; Donaldson & Lindroth, 2007), forest tent caterpillars (Hwang & Lindroth, 1997), and other herbivores (e.g., Wooley, Walker, Vernon, & Lindroth, 2008). In contrast, SPGs are sequestered by keystone, leaf-feeding beetles of the genus *Chrysomela* and used in their own defenses (Soetens, Pasteels, Daloz, & Kaisin, 1998; see also Kearsley & Whitham, 1992; Martinsen, Driebe, & Whitham, 1998; Waltz & Whitham, 1997). Foliar SPGs have also been shown to affect symbiotic microbes. For example, studies of aspen-gypsy moth interactions at the “foliar-gut interface” have shown that SPGs can influence midgut community composition (Mason, Rubert-Nason, Lindroth, & Raffa, 2015). Some midgut bacteria are able to metabolize SPGs (Mason, Lowe-Power, Rubert-Nason, Lindroth, & Raff, 2016) and could provide new models for investigating the evolution of symbioses as driven by plant defensive chemistry.

The examples above suggest that the ability to manipulate SPG gene expression could contribute to a better understanding of the evolution of plant chemical diversity and its consequences for other aspects of *Populus* biology, such as trade-offs between growth and defense (Osier & Lindroth, 2006). Such an understanding is also likely to play a role in the eventual exploitation of plant genes for increased disease resistance, wood quality, herbal flavor and fragrance

products, nutraceuticals, and pharmaceuticals (Tsai, Harding, et al., 2006).

The discovery of SPG genes has been hampered, in part, by their apparent absence in model herbaceous species (Tsai, Harding, et al., 2006). However, forest trees from the model genus *Populus* express a diverse array of SPGs and other secondary metabolites of interest (Boeckler et al., 2011; Chen, Liu, Tschaplinski, & Zhao, 2009; Constabel & Lindroth, 2010; Keefover-Ring et al., 2014). *Populus* species have been studied extensively in genomic (Tuskan et al., 2006), metabolomic (Morreel et al., 2006; Tsai, Harding, et al., 2006; Tsai, Kayal, et al., 2006), ecological (Boeckler et al., 2011; Caseys, Stritt, Glauser, Blanchard, & Lexer, 2015; Lindroth & St. Clair, 2013), and commercial improvement (Jansson & Douglas, 2007; Taylor, 2002; Wullschlegel, Jansson, & Taylor, 2002) research. The North American black cottonwood (*P. trichocarpa*) was the first forest tree to have its genome sequenced (Tuskan et al., 2006), and it and many of its congeners are valuable resources for wood fiber, carbon sequestration, and biofuels development (Taylor, 2002). Across the northern hemisphere, *Populus* species frequently act as foundation species—organisms that modulate and stabilize fundamental community and ecosystem processes (Ellison et al., 2005; Whitham et al., 2006) in riparian and other forest ecosystems. Ease of sexual and vegetative propagation, fast growth rate, short time to sexual maturity, and widespread interspecific hybridization predispose *Populus* to experimental manipulation, and numerous genetic mapping pedigrees have been created to study the genetic basis of many traits of commercial or ecological significance (e.g., DeWoody et al., 2013; Rae et al., 2008; Robinson et al., 2012; Woolbright et al., 2008).

Here, we used a previously published (Woolbright et al., 2008) backcrossed mapping population of naturally hybridizing cottonwoods (i.e., *P. fremontii* and *P. angustifolia*) to identify QTL associated with the SPGs salicortin and HCH-salicortin. While the ecological effects of HCH-salicortin have yet to be established in empirical studies using the purified compound, it differs from salicortin only in the addition of a second hydroxycyclohexane-on-oyl (HCH) functional group (Rehill, Clauss, Wiczorek, Whitham, & Lindroth, 2005). As this functional group confers toxicity to SPGs (Lindroth, Scriber, & Hsia, 1988), HCH-salicortin is likely as biologically active as, or more so than salicortin and related compounds that contain the chemical moiety.

Our study was designed with three long-term objectives in mind: First, we sought to contribute to the discovery of genes controlling an important, but largely uncharacterized metabolic pathway. Second, we aimed to complement other recent studies investigating the evolution of plant secondary chemistry in *Populus* (Caseys et al., 2015; Chedgy et al., 2015; see also Bernhardson et al., 2013). Third, we looked to establish a basis for genomic approaches to community ecology that will link changes at the DNA sequence level with variation in ecologically important chemical traits that influence other species, shape community structure, and drive ecosystem processes (Schuman, Allmann, & Baldwin, 2015). Given patterns of inheritance observed in previous studies (Bailey, Wooley, Lindroth, & Whitham, 2006; Rehill et al., 2005, 2006; Stevens & Lindroth, 2005), we made the following predictions:

1. Expression of salicortin and HCH-salicortin should be correlated given potential precursor–product relationships or competition for shared substrates.
2. Only those progeny that received donor parent (*P. fremontii*) alleles at certain loci would express the trait because HCH-salicortin is expressed at very low levels in some *P. angustifolia* genotypes (Holeski, Hillstrom, Whitham, & Lindroth, 2012) and is not detectable in others, including the recurrent parent genotype in our study.

2 | MATERIALS AND METHODS

2.1 | Pedigree and linkage map

QTL analyses were performed using a previously described mapping pedigree of hybridizing cottonwoods (Woolbright et al., 2008). Briefly, we crossed two individuals (F_1 genotype WSU-6 and *P. angustifolia* genotype #996) from a natural population from the Weber River of northern Utah (Keim et al. 1989, Martinsen et al. 1998) to create a backcross mapping population of 246 backcross progeny. Both parental genotypes were of unknown parentage and were identified using RFLP markers. Progeny were raised under greenhouse conditions at Northern Arizona University's research greenhouse facility. Leaf samples for both genetic and chemical analyses were collected on dry ice from cuttings that were at least 2 years old and propagated in five-gallon pots. Samples were lyophilized, ground in a Wiley mill, and stored at -20°C until use.

The mapping pedigree was used to construct a framework linkage map composed of 326 AFLP markers distributed among nineteen linkage groups (Woolbright et al., 2008). Chromosome assignments and marker order were established using MapMaker 3.0 (Lander et al., 1987) with “default linkage criteria” of LOD = 8.0 and recombination fraction (rf) = 0.37. One hundred eleven microsatellite (SSR) markers conserved across *Populus* species were also included in the complete map allowing us to anchor our linkage groups to the nineteen *Populus* chromosomes (see Tuskan et al., 2006). Thirty-eight AFLP markers were dropped from the QTL analysis due to segregation distortion (Woolbright et al., 2008). In addition, four others were dropped due to unacceptable levels of missing data. Finally, the order of four markers was changed following reanalysis of marker data using the software package R/QTL (Arends, Prins, Jansen, & Broman, 2010; Broman & Sen, 2009, two on chromosome chr2 and two on chromosome chr18). New maps were generated using the *switch.order* function in R/QTL and the entire linkage map for all 19 chromosomes was reestimated using *est.map* function with an error. prob = 0.03 (the estimated scoring error rate for AFLP markers).

2.2 | Chemistry data

Phenolic glycoside concentrations (salicortin and HCH-salicortin) were determined for 172 progeny as per Lindroth, Scriber, & Hsia, 1986. Briefly, 25 mg of freeze-dried leaf powder was placed in 1 ml of methanol at 0°C and sonicated for 30 min. The extract was analyzed by high-performance thin-layer chromatography using salicortin and

HCH-salicortin as standards. These compounds are the major phenolic glycosides in this system (Rehill et al., 2005); standards were purified from cottonwood leaves by liquid–liquid extraction (Lindroth et al., 1996), followed by medium-pressure, “flash” chromatography (Still, Kahn, & Mitra, 1978). Correlation analysis (Spearman's rank) and nonlinear regression (LOESS) of the two traits were performed in R (R Core Team, 2016) and graphed using ggplot2 (Wickham, 2009). Prior to QTL analyses, salicortin data were transformed using the Box–Cox method via online software from Wessa (2016) to establish a normal distribution. All leaf salicortin and HCH-salicortin phenotypic measurements are reported as percent dry weight (%dw).

2.3 | QTL analyses

Salicortin QTL were identified using the MQM (multiple qtl mapping) function of R/QTL. For missing data, the most likely marker genotype was estimated via multiple imputation using the *mqmaugment* feature with the maximum number of augmented genotypes set at 1,024; a minimum probability of 0.30; and strategy = “impute.” For QTL mapping, we used the function *set.cofactors* to generate potential cofactors at 5 cM spacing across the entire linkage map. QTL were identified using the *mqmscan* function with cofactor significance of 0.002. Experiment-wise significant thresholds ($\alpha = 0.05$ and 0.10) were estimated via 1,000 permutations using the *mqmpermutation* function and used to identify significant and suggestive QTL, respectively (see Lander & Kruglyak, 1995). Chromosome-wise significance thresholds ($\alpha = 0.10$; van Ooijen, 1999) were also used to identify suggestive QTL. We further investigated significant QTL identified in the MQM analysis by fitting them to multiple QTL models ($\alpha = 0.10$) using the MIM (multiple interval mapping) function of the WinQTLCartographer software (Wang, Basten, & Zeng, 2012). MIM allowed us to validate QTL peaks from the MQM analysis, refine QTL position and effect, and provided estimates of trait variance explained by each QTL (R^2).

HCH-salicortin is usually absent or at low concentration in most *P. angustifolia* genotypes, including our recurrent parent (genet #996), but is present in nearly all *P. fremontii* and F_1 hybrids studied to date (Holeski et al., 2012; Rehill et al., 2005, 2006). As such, it is possible that differences among *Populus* species largely stem from simple (i.e., Mendelian) modes of inheritance. For example, a backcross toward *P. angustifolia* is predicted to result in a bimodal, “spiked” distribution where approximately one-half of the progeny (those receiving the recurrent parental allele) will fail to express the trait. Our data followed such a distribution (Figure 1) and were not suitable for traditional mapping analyses that require normal distributions. Thus, we used R/qt's *scanone* function with the “2part” nonparametric model for bimodal traits and “upper=FALSE” (indicating a “spike” at the zero value). The experiment-wise LOD threshold for HCH-salicortin was determined by 1,000 permutations. Although HCH-salicortin data were indeed bimodal, we obtained rough estimates of QTL effect and percent trait variance explained by the major HCH-salicortin QTL using the composite interval mapping (CIM) function of the WinQTLCartographer software.

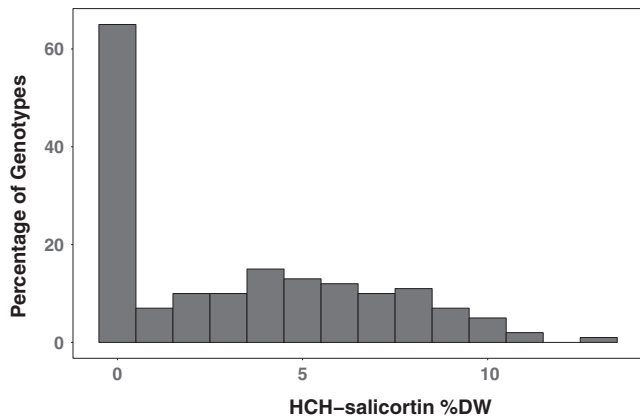


FIGURE 1 HCH-salicortin phenotypes in the mapping population. Histogram of HCH-salicortin measurements showing the “spike” at zero. We searched for QTL using R/qtl’s “2part” model and *scanone* mapping function with “upper=FALSE” for bimodal data with a “spike” at the zero value

2.4 | Candidate genes

Using linear interpolation to align our map with the *Populus trichocarpa* genome sequence (Tuskan et al., 2006), we created lists of candidate genes near each QTL peak. We aligned QTL intervals with the genome sequence (V.3.0) using BLASTN (Altschul, Gish, Miller, Myers, & Lipman, 1990) to determine the physical locations of syntenic SSR markers. Specific criteria for accepting SSR positions were hits with a minimum length of seventeen base pairs, seventy percent (0.70) minimum identity, and agreement with expected SSR product size. Using that information, we aligned the physical positions of SSR markers with their positions in the framework map (Woolbright et al., 2008), and used regression to estimate the physical locations of each QTL interval. When QTL intervals included chromosome ends, we used either zero or total chromosome lengths (cm vs. bp) for the relevant flanking positions.

Gene lists were created using the *Populus trichocarpa* V3.0 sequence and the Biomart function on the Phytozome v12.1 database (Goodstein et al., 2012). We compiled lists from the 2.6-Mb intervals centered on each QTL peak which corresponded to the average ± 1 -LOD “drop” for the significant QTL (approximately 15 cM). By definition, LOD scores for suggestive QTL are relatively low and can result in LOD drops that are prohibitively large. Using the *P. trichocarpa* genome to create gene lists for a map based on *P. fremontii* (our donor parent) presented challenges that are discussed further below.

3 | RESULTS

3.1 | Salicortin

Leaf salicortin levels in the experimental backcrosses differed by 14-fold, ranging from 1.9 to 26.8 percent dry weight (% dw), with an average of 14.1% dw ($\sigma = 4.94$). After Box-Cox transformation to establish a normal distribution, multiple QTL mapping (MQM) revealed

ten significant ($\text{LOD} \geq 2.67$, $\alpha = 0.05$) peaks for salicortin (Figure 2), and one ($\text{LOD} \geq 3.34$) for HCH-salicortin (Figure 3). However, we chose to treat these as seven QTL because the +/1 1.3 Mb flanking sequences used to create our 2.6-Mb intervals overlapped for several peaks (see Table 1). In addition, we identified two suggestive QTL for salicortin ($\text{LOD} \geq 2.40$) using MQM mapping ($\alpha = 0.10$) and six using van Ooijen’s (1999) chromosome-wise method ($\text{LOD} \geq 1.8$, $\alpha = 0.10$). An additional six QTL for HCH-salicortin were also identified using the chromosome-wise threshold ($\text{LOD} \geq 1.8$). QTL were distributed across 14 chromosomes. Data for significant peaks are summarized in Table 1. Five of the eleven significant QTL peaks were retained by the MIM model and are summarized in Table 2. For those five, R^2 values ranged from .03 to .13, and QTL effect varied where substitution of a *P. fremontii* allele resulted in a decrease in salicortin for three QTL and an increase for the remaining two. When both significant and suggestive QTL were included, substitution of *P. fremontii* alleles varied in effect, with nine QTL showing an increase in the trait, and nine showing a decrease. QTL effects ranged from 1.9% dw to -2.34% dw.

Suggestive QTL are summarized in Table 3 (only the sign of the QTL effect is included). Estimates of effect and R^2 were not calculated for suggestive QTL given inherent difficulty with fitting large numbers of QTL to MIM models and the fact that estimates based on suggestive QTL are less likely to be accurate (see Beavis, 1994). Tables 1 and 3 also include SSR markers that are shared with the association study by Caseys et al. (2015).

3.2 | HCH-salicortin

HCH-salicortin ranged from 0.0 to 13.3% dw in the experimental backcross, with an average of 3.4% dw and a standard deviation of 3.5. Supporting our second prediction, we identified a major QTL on chromosome chr12 ($\text{LOD} = 23.4$) that colocalized with a salicortin QTL and that explained at least 52% of the variance. In addition, we identified six suggestive QTL ($\text{LOD} \geq 1.8$, Table 3). Substitution of *P. fremontii* alleles varied in the direction of effect, with an average increase in HCH-salicortin for the major QTL on chr12. For the six suggestive QTL, three resulted in an increase in the trait, and three resulted in a decrease.

There was no simple linear relationship between salicortin and HCH-salicortin as illustrated in Figure 4. While a Spearman’s rank test showed that salicortin and HCH-salicortin were negatively correlated ($r_s = -0.2903$, $p < .0001$), a LOESS line (nonlinear regression) fitted to the data illustrates complexity in the relationship where the two traits are positively correlated from low- to midrange values, but are negatively correlated when salicortin levels are highest. However, note that several individuals expressed both traits at high values.

3.3 | Gene lists

The total number of genes and transcripts in our 2.6 Mb QTL intervals was 4,463, representing approximately 10.8% of the total genes (41, 335) for the *Populus* V3.0 sequence (see Sundell et al., 2015).

TABLE 1 Results of MQM analyses. Peaks were identified using R/qtl's MQM (salicortin) and "2-part" (HCH-salicortin) models ($\alpha = 0.05$). LOD thresholds were ≥ 2.67 for salicortin and ≥ 3.34 for HCH-salicortin. QTLs are named for the chromosome on which they occur and the order in which they were discovered. For example, QTL Sal_4.2 represents the second QTL described for chr04. Pos(cM) = position on the linkage map. Pos (Mb) = position in the genome. LOD is the maximum LOD score at the QTL peak. Column labeled "a" indicates the phenotypic effect (increase or decrease of the trait value) when the donor parent (*P. fremontii*) allele is substituted for the recurrent parent (*P. angustifolia*) allele at each QTL. QTL from Caseys et al. (2015) and the SPGs they correspond to were included when they occurred on the same chromosomes. Associated SSR is the marker nearest the QTL described by Caseys et al. (2015) and pos(Mb) is that marker's position in the v3.0 genome. Periods (".") denote data on associated marker or QTL

| QTL | pos (cM) | pos (Mb) | LOD | a | <i>P. tremula</i> and <i>P. alba</i> QTL (Caseys et al., 2015) | Associated SSR | Pos (Mb) |
|----------|----------|----------|-------|---|--|----------------|----------|
| Sal_2.1 | 62.50 | 8.77 | 3.14 | + | . | . | . |
| Sal_5.1 | 22.63 | 3.77 | 4.82 | - | . | . | . |
| Sal_5.2 | 79.79 | 15.81 | 3.33 | + | . | . | . |
| Sal_5.3 | 100.60 | 20.58 | 5.41 | + | Tremuloidin, tremulacin isomer | W15 | 25.78 |
| Sal_7.1 | 0 | 0 | 3.04 | - | Salicortin Isomer 2 | O312 | 3.35 |
| Sal_12.1 | 74.00 | 13.08 | 18.86 | - | Acetyl-salicortin isomer 1, Isomer 2 | G1186 | 12.95 |
| Sal_12.2 | 77 | 14.00 | 19.89 | - | Acetyl-salicortin isomer 1, Isomer 2 | G1186 | 12.95 |
| HCH_12.1 | 82.66 | 14.94 | 25.53 | + | Acetyl-salicortin isomer 1, Isomer 2 | G1186 | 12.95 |
| Sal_15.1 | 46.80 | 9.32 | 6.17 | + | . | . | . |
| Sal_15.2 | 52.57 | 10.43 | 5.99 | + | . | . | . |
| Sal_15.3 | 58.49 | 11.58 | 5.33 | + | . | . | . |

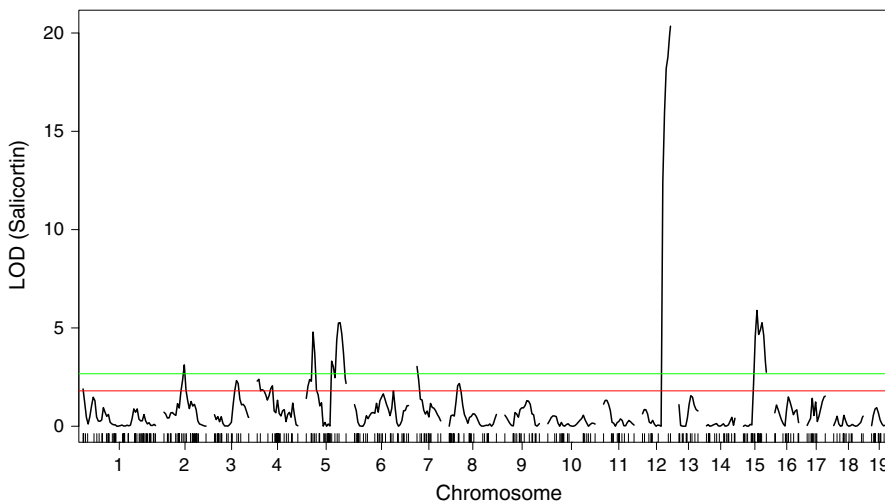


FIGURE 2 LOD scores for salicortin at all nineteen chromosomes. Horizontal lines represent the experiment-wise (green, $\alpha = 0.05$) and chromosome-wise suggestive (red, $\alpha = 0.10$) QTL thresholds

Our lists include genes models that lack general identifiers or descriptions. When relevant, the positions of SSR markers used in the study by Caseys et al. (2015) were also included. Table S1 provides total chromosome-wise gene lists and includes locations of our QTL peaks and SSRs from Caseys et al. (2015) as red text.

We identified eight genes that are associated with secondary metabolites (primarily hydroxycinnamate precursors and derivatives) and thought to play roles in predicted steps in the SPG pathway (see Tsai et al., 2006a,b) or similar metabolic pathways (see Bonpart et al., 2015). Candidate genes are summarized on Table S2 and include two BAHD-type acyltransferases, eight *cinnamoyl-coA reductases*, and *Shikimate dehydrogenase*. Supplemental tables are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.8h67t>.

4 | DISCUSSION

In this study, we identify several QTL associated with the SPGs salicortin and HCH-salicortin. The most noteworthy QTL occur at the distal end of chr12 and overlap for the two traits. The major QTL for HCH-salicortin on chr12 represent a likely Mendelian locus, and, as such, it could prove to be an important starting point for identifying undescribed SPG genes. Our results also complement other recent studies focusing on association mapping of metabolomic data (Caseys et al., 2015) including gene expression and function of candidate genes associated with SPG synthesis (Chedgy et al., 2015; see also Tsai et al., 2016a,b). Data from our study create foundations for verifying the results of future investigations of the genetic basis of SPG synthesis and the ultimate discovery of SPG genes.

TABLE 2 QTL retained in the MIM Model. MQM QTL peaks that were also supported by MIM model-fitting ($\alpha = .10$). Columns are as in Table 1, with the addition of a column for the R^2 value for each QTL as estimated by MIM mapping

| QTL | pos (cM) | LOD | a | R^2 | <i>P. tremula</i> and <i>P. alba</i> QTL (Caseys et al., 2015) | Associated SSR |
|----------|----------|------|------|-------|--|----------------|
| Sal_2.1 | 53.0 | 3.23 | 1.96 | .09 | . | . |
| Sal_5.3 | 96.4 | 2.68 | 1.90 | .06 | Tremuloidin, tremulacin isomer | W15 |
| Sal_7.1 | 0.0 | 2.19 | 1.81 | .06 | Salicortin Isomer 2 | O312 |
| Sal_12.1 | 58.9 | 6.07 | 2.35 | .13 | Acetyl-salicortin isomer 1, Isomer 2 | G1186 |
| Sal_15.1 | 46.8 | 1.32 | 1.57 | .043 | . | . |

TABLE 3 Suggestive QTL from the MQM analyses and chromosome-wise thresholds. Data are arranged as per Table 1, but for suggestive rather than significant peaks

| QTL | pos (cM) | Pos (Mb) | LOD | a | <i>P. tremula</i> and <i>P. alba</i> QTL (Caseys et al., 2015) | Associated SSR | Pos (Mb) |
|----------|----------|----------|------|---|--|----------------|----------|
| Sal_1.1 | 0.00 | 0.00 | 1.90 | + | . | . | . |
| HCH_1.1 | 10.40 | 2 | 1.87 | + | . | . | . |
| Sal_3.1 | 69.50 | 16.14 | 2.39 | - | Acetyl-salicortin, isomer 1, isomer 2; tremuloidin; tremuloidin; tremulacin isomer | G1869 | 15.28 |
| Sal_4.1 | 6.84 | 1.62 | 2.45 | - | . | . | . |
| Sal_4.2 | 44.91 | 6.54 | 2.06 | - | Salicortin isomer 3 | O127 | 6.64 |
| Sal_5.4 | 12.02 | 2.24 | 2.40 | - | . | . | . |
| HCH_5.1 | 16.20 | 2.84 | 2.31 | - | . | . | . |
| HCH_5.2 | 26.56 | 4.34 | 2.65 | - | . | . | . |
| Sal_6.1 | 112.62 | 23.10 | 1.93 | - | HCH-tremulacin | O369 | 23.66 |
| Sal_8.1 | 31.92 | 3.70 | 2.34 | + | HCH-Salicortin | O374 | 6.69 |
| HCH_11.1 | 56.00 | 10.83 | 2.44 | + | Tremulacin | G1037 | 6.99 |
| Sal_17.1 | 20.52 | 3.11 | 1.97 | + | . | . | . |
| HCH_18.1 | 76.62 | 15.03 | 2.91 | - | . | . | . |
| HCH_19.1 | 45.10 | 10.4 | 2.22 | + | . | . | . |

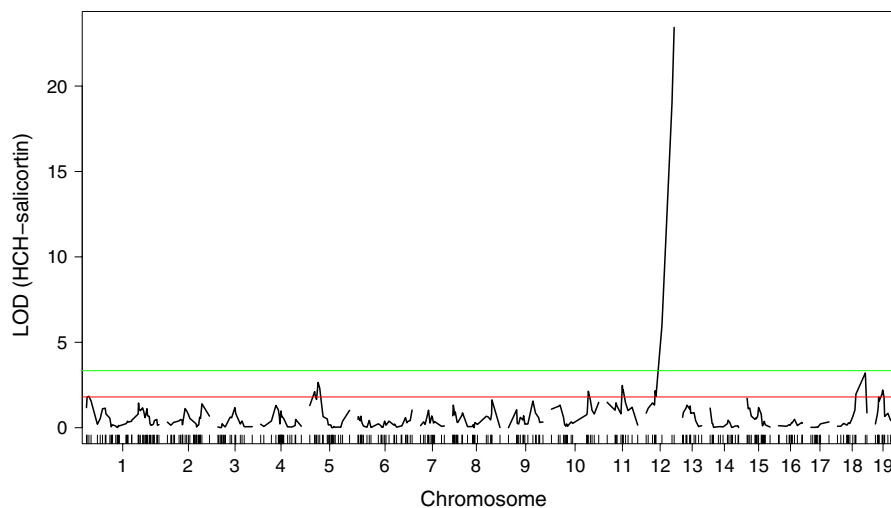


FIGURE 3 LOD scores for HCH-salicortin at all nineteen chromosomes. Horizontal lines represent the experiment-wise (green, $\alpha = 0.05$) and chromosome-wise suggestive (red, $\alpha = 0.10$) QTL thresholds

4.1 | Identification of suggestive versus significant QTL

Choice of mapping strategy can influence the identification of QTL, especially where small populations are involved. We chose to search for suggestive QTL using relaxed LOD thresholds ($\alpha = 0.10$) for both the MQM mapping (Arends et al., 2010; Broman & Sen, 2009) and chromosome-wise threshold (van Ooijen, 1999) methods in order to increase the number of QTL identified. That choice, as well as the failure to retain all loci in the MIM model, resulted in 14 of 21 QTL being categorized as “suggestive” rather than “significant” (see Lander & Kruglyak, 1995). In the past, concern that erroneous QTL (i.e., type I errors) would lead to “bloated literature” has led to relatively conservative suggestions for setting QTL thresholds (see Lander & Kruglyak, 1994). However, given the exploratory nature of most QTL studies, Beavis (1998) noted that, in the long run, type II errors (missing QTL) might be costlier than type I errors (false QTL; see also Holland, 2005), despite his own work demonstrating the increased risk of type I errors when dealing with small populations (i.e., the so-called Beavis Effect). Likewise, Lander & Kruglyak (1994) did not completely dismiss suggestive QTL, but called for caution when reporting them. Today, when whole genomes and massively parallel sequencing make it possible to assess entire transcriptomes (e.g., RNA-seq), virtually any QTL might prove useful if it helps to validate gene expression data. In keeping with the standards set by Lander and Kruglyak (1995), we acknowledge the “tantalizing but unproven” nature of our suggestive QTL, with an eye toward ongoing and future genomic and transcriptomic research in *Populus*. Thus, we chose to include *all* intervals and their corresponding gene lists in this article.

4.2 | Trait variation and correlation between salicortin and HCH-salicortin

For overlapping QTL intervals, *P. fremontii* alleles resulted in decreased values for both traits for the QTL on chr05. In contrast, substitution of *P. fremontii* alleles at our largest QTL (sal 12 and HCHsal 12) increased HCH-salicortin but decreased salicortin. While this could be explained by precursor-product or competitive relationships, the lack of a linear correlation between the two traits (Figure 4) prevents us from accepting our first prediction that the two traits would be correlated in a manner explicable by our QTL data (e.g., by the conversion of salicortin to HCH-salicortin as governed by a QTL such as occurs on chr12). Instead, it appears that the relationship between the two traits is complex and understanding their relative fluxes requires a broader metabolomic perspective that was outside the scope of our study (but see Tsai et al., 2006a; Casey's et al., 2015). To date, the SPG pathway remains poorly described, underscoring the need for a genomic basis of SPG synthesis and flux.

Variation in the sign of the QTL effects for each trait could result from species-level differences (e.g., preferred pathways, substrate specificity, allelic, or isozyme efficiency) or from similar

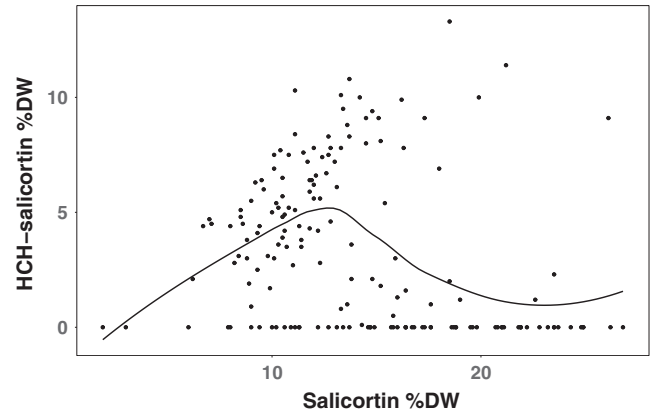


FIGURE 4 Salicortin and HCH-salicortin correlation. A LOESS line fitted to the raw salicortin and HCH-salicortin measurements across all individuals illustrates the complex relationship between the two traits

allelic variation within the wild (outcrossed) parental genotypes. On average, salicortin levels are higher in *P. angustifolia* than *P. fremontii*; however, they vary considerably within and among species and hybrids (Rehill et al., 2005, 2006). Despite the overlap in salicortin levels, we were able to identify QTL potentially reflecting allelic or isozyme differences between hybridizing *Populus* species. In contrast, the fact that *P. angustifolia* does not produce HCH-salicortin allowed us to identify six potential QTL for HCH-salicortin, including a major locus on chr12 where substitution of a *P. fremontii* allele appears to be required for expression of the trait (thus supporting Prediction 2, above). The concentration of leaf HCH-salicortin differs significantly among *Populus* generic sections, and our QTL data complement the only other published study that identifies QTL intervals associated with SPGs, including salicortin and HCH-salicortin (Caseys et al., 2015). Likewise, our HCH-salicortin QTL data support one other study that investigated specific candidate genes that could be directly involved with SPG synthesis (Chedgy et al., 2015; discussed below).

4.3 | Candidate genes

Our study was intended to generate preliminary data on regions of the *Populus* genome associated with SPG synthesis. Given the length of our QTL intervals, the candidate genes discussed here are informed speculation at best, and so conclusions should be treated with caution. However, our choice to further discuss specific genes is supported by previous studies. For example, starting with the strongest QTL on chr12, we searched for candidate genes that were orthologous with other plant genes controlling similar metabolic pathways, especially those that have been tentatively linked to proposed steps within the *Populus* SPG pathway (Chedgy et al., 2015; Tsai, Harding, et al., 2006; Tsai, Kayal, et al., 2006). Our search revealed two hydroxycinnamoyl/benzoyltransferases that belong to the BAHD-type acyltransferase family of genes Potri.001G042900 and Potri.012G144500. The first (on chr01) encodes HCT6 which is

associated with lignin biosynthesis in vascular tissues (Touminen, Johnson, & Tsai, 2011; Tsai, Harding, et al., 2006; Tsai, Kayal, et al., 2006; Wegrzyn et al., 2010) and has not been shown to affect SPGs. In contrast, the function of Potri12G144500, which occurs within the interval for our strongest QTL (on chr12), remains undescribed. However, others have noted similar N-hydroxycinnamoyl/benzoyl-transferases that have substrates or products that are also thought to play roles in SPG expression via control over the synthesis of benzyl benzoate and salicyl benzoate—possible intermediates in the SPG pathway (Chedgy et al., 2015; see also Bapst, Harding, & Tsai, 2010). Interestingly, Potri.012G144500 is one of the final fourteen transcripts identified for that end of chromosome chr12 under the current (V3.0) annotated genome sequence. While MQM mapping resulted in slight map expansion due to the introduction of pseudomarkers, CIM of the original, nonimputed maps placed QTL peaks for salicortin and HCH-salicortin at terminus of the linkage map for chromosome chr12—which provides intriguing, albeit circumstantial support for candidate gene status. These findings are important because Chedgy et al. (2015) showed that enzyme activity of similar genes (e.g., *PtACT49* on chromosome chr19) is consistent with hypothesized steps in the SPG pathway (see Tsai, Harding, et al., 2006; Tsai, Kayal, et al., 2006).

Several other candidate genes identified in our study are linked to pathways that might be coregulated or otherwise linked with SPG synthesis. Examples include *shikimate dehydrogenase* (chr05) which catalyzes the conversion of 3-dehydroshikimate to shikimate—a key entry point to the shikimic acid pathway (aka phenylpropanoid pathway). That pathway eventually leads to production of phenylalanine, which is deaminated to produce cinnamic acid—very likely an initial precursor for PGs (Tsai, Kayal, et al., 2006). Eight *cinnamoyl CoA-reductase* (*CCR*) genes were found on (chr01). Cinnamic acid is thought to be one of the likely starting points of the SPG pathway (Tsai, Harding, et al., 2006; Tsai, Kayal, et al., 2006). Cinnamic acid leads to production of benzoyl-CoA, which can be catalyzed by *CCR* to produce benzaldehyde. Benzaldehyde can then be metabolized to salicylaldehyde which is also one of the several precursor compounds (another being salicylic acid) that could lead to production of SPGs. While these compounds and genes create potential starting points for understanding SPG metabolism, it is important to emphasize that the main pathway(s) leading to remains unresolved.

4.4 | Alignment with the *P. trichocarpa* genome

Creating candidate gene lists was challenging given the lack of a genome sequence for our parent species. This was particularly true for *P. fremontii*, our donor parent and source of the marker alleles used to create our map. While *P. angustifolia* is from the same generic section as *P. trichocarpa* (sect. *Tacamahaca*), *P. fremontii* is within a different section (sect. *Aigeiros*). However, previous studies have shown high levels of synteny among *Populus* species from different generic sections, as well as a high transferability of SSR markers (Berlin et al., 2010). Still, small-scale differences between our map and *P. trichocarpa* have been noted (see Woolbright et al., 2008) and

could be the product of real differences among species/sections or the result of typical mapping errors—especially those associated with relatively small sample sizes. Thus, in order to choose the size of the interval used to search for candidate genes, we had to balance the need for concise gene lists with the possibility that our QTL intervals did not align precisely with the reference genome. We created gene lists from physical intervals of 2.6 Mb, which corresponded to the average ± 1 -LOD drop around our significant QTL peaks. In addition, two of our intervals contained SSR markers shared with the recent SPG association mapping study of Caseys et al. (2015). This supports the notion that these loci could be useful starting points for future genetic and genomic studies of SPG synthesis, especially those employing expression-based approaches such as eQTL mapping, RNA-seq and knockdown/out/up studies.

4.5 | Ecological significance

Poplars are foundation trees, and population-level variation in leaf chemistry is one of the strongest drivers of associated arthropod community organization (Bangert et al., 2006; Bernhardson et al., 2013; Martinsen et al., 2006). Variation in the distribution and abundance of foundation arthropods can, in turn, affect multitrophic-level interactions with consequences throughout associated communities and ecosystems (Keith, Bailey, Lau, & Whitham, 2017; Shuster, Lonsdorf, Wimp, Bailey, & Whitham, 2006; Whitham et al., 1999, 2006, 2008). Knowing which genes and alleles drive these patterns is a major goal in the fields of community and ecosystem genetics (Whitham et al., 2008), and the genes regulating SPGs are prime candidates for realizing that goal. For example, phenolic glycosides are important defenses against a variety of foundation defoliators including gypsy moths and forest tent caterpillars (Hwang & Lindroth, 1997), but are beneficial to chrysomelid beetles (*Chrysomela confluenta*) that sequester the plant's defenses for their own defense (Martinsen et al., 1998). The enzymatic steps linking leaf salicortin with beetle sequestered defensive chemistry have been described (Pasteels, Rowell-Rahier, Braekman, & Dupont, 1991), and future discovery of *Populus* phenolic glycoside genes should reveal a metabolic pathway that originates in a foundation plant and continues in a foundation herbivore whose distribution and abundance can scale up to affect entire communities (Waltz & Whitham, 1997).

5 | CONCLUSIONS

While our results are preliminary steps toward identifying SPG genes, our study has five important conclusions. First, it is the first study of salicinoid phenolic glycosides to use a full-sib mapping population and a linkage map anchored to the *Populus* genome sequence to identify QTL interval for salicinoid phenolic glycosides. Second, it complements a recent association mapping study by Caseys et al. (2015) that used European *Populus* species to investigate the

evolution of SPG diversity, and constraints therein, for a more distantly related section of the genus. Third, we identify a major likely Mendelian locus for HCH-salicortin that provides a focal point for future research on SPG gene discovery. Fourth, our QTL intervals contain candidate genes known to be associated with compounds that are thought to play potential roles in the SPG pathway (i.e., hydroxycinnamoyl and its precursors or derivatives; Tsai, Harding, et al., 2006; Tsai, Kayal, et al., 2006) and are similar to those discussed by Chedgy et al. (2015). Finally, our results are an important first step toward linking genomic data with community-level patterns—a major goal in the developing field of community genetics and evolution (e.g., Allen et al., 2012; Whitham et al., 2006, 2008). These conclusions emphasize the role of *Populus* as a model organism for studying the chemical ecology and evolution of foundation forest trees and for the advancement of the developing field of community genetics and genomics.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of the following individuals: Phil Service, Tom Sisk, Jen Schweitzer, Joe Bailey, Celine Caseys, Christian Lexer, Justin McGrath, Karl Broman, Danny Arends, Stephanie Woolbright, and Tricia Covington. Special thanks to Brad Blake and Phil Patterson (NAU Greenhouse); the students, faculty, and staff of the NAU Department of Biological Sciences; the NAU Cottonwood and Pinyon Ecology Groups; and the Postdoctoral Fellows Program and the Genomic Ecology of Global Change (GEGC) theme at the University of Illinois Carl R. Woese Institute for Genomic Biology (IGB). Funding and other support were provided by the UA Little Rock Department of Biology and College of Arts, Letters, and Sciences; the Phoenix chapter of the ARCS Foundation; NSF FIBR grant DEB-0425908, NSF MacroSystems grant DEB-1340852, and NSF DBI-1126840 for establishing the Southwest Experimental Garden Array.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

TGW, PK, GDM, and SAW planned and designed the experiments; RLL and BJR conducted the chemical analyses and contributed to writing and editing; GDM and SAW conducted the experimental crosses and performed the QTL experiments; SPD and MSZ conducted genomic data analyses, and participated in writing and editing; GJA oversaw laboratory work and contributed to data analysis, writing, and editing; SAW wrote the manuscript.

ORCID

Scott A. Woolbright  <http://orcid.org/0000-0002-7886-1009>

REFERENCES

- Allen, G. J., Shuster, S. M., Woolbright, S. A., Walker, F., Meneses, N., Keith, A., & Whitham, T. G. (2012). Perspective: Interspecific indirect genetic effects (IIEGs). Linking genetics and genomics to community ecology and ecosystem processes. In T. Ohgushi, O. J. Schmitz, & R. D. Hold (Eds.), *Trait-mediated indirect interactions: Ecological and evolutionary perspectives* (pp. 295–323). New York, NY: Cambridge University Press.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
- Arends, D., Prins, P., Jansen, R. C., & Broman, K. W. (2010). R/qtl: High-throughput multiple QTL mapping. *Bioinformatics*, 26, 2990–2992. <https://doi.org/10.1093/bioinformatics/btq565>
- Bailey, J. K., Wooley, S. C., Lindroth, R. L., & Whitham, T. G. (2006). Importance of species interactions to community heritability: A genetic basis to trophic-level interactions. *Ecology Letters*, 9, 78–85.
- Bangert, R. K., Turek, R. J., Rehill, B., Wimp, G. M., Schweitzer, J. A., Allan, G. J., ... Whitham, T. G. (2006). A genetic similarity rule determines arthropod community structure. *Molecular Ecology*, 15, 1379–1391. <https://doi.org/10.1186/1471-2164-11-129>
- Bapst, B., Harding, S., & Tsai, C.-J. (2010). Biosynthesis of phenolic glycosides from phenylpropanoid and benzenoid precursors in *Populus*. *Journal of Chemical Ecology*, 36, 286–297. <https://doi.org/10.1007/s10886-010-9757-7>
- Beavis, W. D. (1994). The power and deceit of QTL experiments: lessons from comparative QTL studies. *Proceedings of the forty-ninth annual corn and sorghum industry research conference*, pp. 250–266.
- Beavis, W. D. (1998). QTL analyses: Power, precision, and accuracy. In A. H. Paterson (Ed.), *Molecular dissection of complex traits* (pp. 145–162). Boca Raton, FL: CRC Press.
- Bernhardson, C., Robinson, K. M., Abreu, I. N., Jansson, S., Albrechtsen, B. R., & Ingvarsson, P. K. (2013). Geographic structure in metabolome and herbivore community co-occurs with genetic structure in plant defense genes. *Ecology Letters*, 16, 791–798. <https://doi.org/10.1111/ele.12114>
- Berlin, S., Lagercrantz, U., von Arnold, S., Öst, T., & Rönnerberg-Wästljung, A. C. (2010). High-density linkage mapping and evolution of paralogs and orthologs in *Salix* and *Populus*. *BMC Genomics*, 14, 129.
- Boeckler, A., Gershezon, J., & Unsicker, S. B. (2011). Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. *Phytochemistry*, 72, 1497–1509. <https://doi.org/10.1016/j.phytochem.2011.01.038>
- Bontpart, T., Cheynier, V., Ageorges, A., & Terrier, N. (2015). BAHD or SCPL-acyltransferase? What a dilemma for acylation in the world of plant phenolic compounds. *New Phytologist*, 208, 695–707. <https://doi.org/10.1111/nph.13498>
- Broman, K. W., & Sen, S. (2009). *A guide to QTL mapping with R/qtl*. New York, NY: Springer. <https://doi.org/10.1007/978-0-387-92125-9>
- Caseys, C., Stritt, C., Glauser, G., Blanchard, T., & Lexer, C. (2015). Genetic architecture of phenylpropanoids in European *Populus* species. *PLoS ONE*, 10, e-128200. <https://doi.org/10.1371/journal.pone.0128200>
- Chedgy, R. J., Köllner, T. G., & Constabel, C. P. (2015). Functional characterization of two acyltransferases from *Populus trichocarpa* capable of synthesizing benzyl benzoate and salicyl benzoate, potential intermediates in salicinoid phenolic glycoside biosynthesis. *Phytochemistry*, 113, 149–159. <https://doi.org/10.1016/j.phytochem.2014.10.018>
- Chen, F., Liu, C. J., Tschaplinski, T. J., & Zhao, N. (2009). Genomics of secondary metabolism in *Populus*: Interactions with biotic and abiotic environments. *Critical Reviews in Plant Sciences*, 28, 375–392. <https://doi.org/10.1080/07352680903241279>

- Constabel, P. C., & Lindroth, R. L. (2010). The impact of genomics on advances in herbivore defense and secondary metabolism in *Populus*. In S. Jansson, R. Bhalerao, & A. Groover (Eds.), *The genetics and genomics of populus* (pp. 279–305). New York, NY: Springer. <https://doi.org/10.1007/978-1-4419-1541-2>
- Couture, J. J., Singh, A., Rupert-Nason, K. F., Serbin, S. P., Lindroth, R. L., & Townsend, P. A. (2016). Spectroscopic determination of ecologically relevant plant secondary metabolites. *Methods in Ecology and Evolution*, 7, 1402–1412. <https://doi.org/10.1111/2041-210X.12596>
- Devore, E. E., Kang, J. H., Breteler, M. M. B., & Grodstein, F. (2013). Dietary intake of berries and flavonoids in relation to cognitive decline. *Annals of Neurology*, 72, 135–143.
- DeWoody, J., Viger, M., Lakatos, F., Tuba, K., Taylor, G., & Smulders, M. (2013). Insight into the genetic components of community genetics: QTL mapping of insect association in a fast-growing forest tree. *PLoS ONE*, 8, e79925. <https://doi.org/10.1371/journal.pone.0079925>
- Donaldson, J. R., & Lindroth, R. L. (2007). Genetics, environment, and their interaction determine efficacy of chemical defense in trembling aspen. *Ecology*, 88, 729–739. <https://doi.org/10.1890/06-0064>
- Ellison, A. M., Bank, M. S., Clinton, B. D., Colburn, E. A., Elliott, K., Ford, C. R., ... Mohan, J. (2005). Loss of foundation species: Consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment*, 3, 479–486. [https://doi.org/10.1890/1540-9295\(2005\)003\[0479:LOFSCF\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2005)003[0479:LOFSCF]2.0.CO;2)
- Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., ... Rokhsar, D. S. (2012). Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Research*, 40, <https://doi.org/10.1093/nar/gkr944>
- Harbilas, D., Vallerand, D., Brault, A., Ammar, S., Arnason, J. T., Musallam, L., & Haddad, P. S. (2013). *Populus balsamifera* extract and its active component salicortin reduce obesity and attenuate insulin resistance in a diet-induced obese mouse model. *Evidence-Based Complementary and Alternative Medicine*, 2013, <https://doi.org/10.1155/2013/172537>
- Holeski, L. M., Hillstrom, M. L., Whitham, T. G., & Lindroth, R. L. (2012). Relative importance of genetic, ontogenetic, induction and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. *Oecologia*, 170, 695–707. <https://doi.org/10.1007/s00442-012-2344-6>
- Holeski, L. M., McKenzie, S. C., Kruger, E. L., Couture, J. J., Rupert-Nason, K., & Lindroth, R. L. (2016). Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (*Populus tremuloides*) to browsing by a keystone forest ungulate. *Journal of Ecology*, 104, 850–863. <https://doi.org/10.1111/1365-2745.12559>
- Holland, J. (2005). QTL mapping II. [WWW document]. Retrieved from <http://www4.ncsu.edu/~jholland/CS746/QTLMapping2.pdf> [accessed 27 December 2012].
- Hwang, S. Y., & Lindroth, R. L. (1997). Clonal variation in foliar chemistry of aspen: Effects on gypsy moths and forest tent caterpillars. *Oecologia*, 111, 99–108. <https://doi.org/10.1007/s004420050213>
- Jansson, S., & Douglas, C. J. (2007). *Populus*: A model system for plant biology. *Annual Review of Plant Biology*, 58, 435–458.
- Kearsley, M. J. C., & Whitham, T. G. (1992). Guns and butter: a no-cost defense against predation for *Chrysomela confluenta*. *Oecologia*, 92, 556–562.
- Keefover-Ring, K., Ahnlund, M., Abreu, I. N., Jansson, S., Moritz, T., & Albrechtsen, B. R. (2014). Salicinoid chemotypes show no evidence of geographical structure in *Populus tremula*. *PLoS ONE*, 9, e107189. <https://doi.org/10.1371/journal.pone.017189>
- Keim, P., Paige, K. N., Whitham, T. G., & Lark, K. G. (1989). Genetic analysis of an interspecific hybrid swarm of *Populus*: Occurrence of unidirectional introgression. *Genetics*, 123, 557–565.
- Keith, A. R., Bailey, J. K., Lau, M. K., & Whitham, T. G. (2017). Genetics-based interactions of foundation species affect community diversity, stability and network structure. *Proceedings of the Royal Society B*, 284, 20162703. <https://doi.org/10.1098/rspb.2016.2703>
- Kwon, D.-J., Bae, Y.-S., Ju, S. M., Youn, G. S., Soo, Y. C., & Park, J. (2014). Salicortin suppresses lipopolysaccharide-stimulated inflammatory responses via blockade of Ne-KB and JNK activation in RAW 264.7 macrophages. *BMB Reports Online*, 46, 318. <https://doi.org/10.5483/BMBRep.2014.47.6.200>
- Lamit, L. J., Busby, P. E., Lau, M. K., Compson, Z. G., Wojtowicz, T., Keith, A. R., ... Whitham, T. G. (2015). Tree genotype mediates covariance among diverse communities from microbes to arthropods. *Journal of Ecology*, 103, 840–850. <https://doi.org/10.1111/1365-2745.12416>
- Lander, E., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., & Newberg, L. A. (1987). MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, 1, 174–181. [https://doi.org/10.1016/0888-7543\(87\)90010-3](https://doi.org/10.1016/0888-7543(87)90010-3)
- Lander, E., & Kruglyak, L. (1995). Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nature Genetics*, 11, 241–247. <https://doi.org/10.1038/ng1195-241>
- Lee, M., Lee, S. H., Kang, J., Yang, H., Jeong, E. J., Kim, H. P., ... Sung, S. H. (2013). Salicortin-derivatives from *Salix pseudo-lasiogyne* twigs inhibit adipogenesis in 3T3-L1 cells via modulation of C/EBP α and SREBP1c dependent pathway. *Molecules*, 18, 10484–10496. <https://doi.org/10.3390/molecules180910484>
- Lindroth, R. L., Scriber, J. M., & Hsia, M. T. S. (1986). Differential responses of tiger swallowtail subspecies to secondary metabolites from tulip tree and quaking aspen. *Oecologia*, 70, 13–19. <https://doi.org/10.1007/BF00377106>
- Lindroth, R. L., & St. Clair, S. B. (2013). Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. *Forest Ecology and Management*, 299, 14–21. <https://doi.org/10.1016/j.foreco.2012.11.018>
- Martinsen, G. D., Driebe, E. M., & Whitham, T. G. (1998). Indirect interactions mediated by changing plant chemistry: Beaver browsing benefits beetles. *Ecology*, 79, 192–200. [https://doi.org/10.1890/0012-9658\(1998\)079\[0192:IMBCP\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[0192:IMBCP]2.0.CO;2)
- Mason, C. J., Lowe-Power, T. M., Rubert-Nason, K. F., Lindroth, R. L., & Raff, K. F. (2016). Interactions between bacteria and aspen defense chemicals at the phyllosphere–herbivore interface. *Journal of Chemical Ecology*, 42, 193–201. <https://doi.org/10.1007/s10886-016-0677-z>
- Mason, C. J., Rubert-Nason, K. F., Lindroth, R. L., & Raffa, K. F. (2015). Aspen defense chemicals influence midgut bacterial community composition of gypsy moth. *Journal of Chemical Ecology*, 41, 75–84. <https://doi.org/10.1007/s10886-014-0530-1>
- Morreel, K., Goeminne, G., Storme, V., Sterck, L., Ralph, J., Coppieters, W., ... Boerjan, W. (2006). Genetical metabolomics of flavonoid biosynthesis in *Populus*: A case study. *The Plant Journal*, 47, 224–237. <https://doi.org/10.1111/j.1365-313X.2006.02786.x>
- Neuman, K.-H., Kumar, A., & Imani, J. (2009). *Plant cell and tissue culture – A tool in biotechnology, principles and practice*. Berlin, Germany: Springer.
- Osier, T. L., & Lindroth, R. L. (2006). Genotype and environment determine allocation to and costs of resistance in quaking aspen. *Oecologia*, 148, 293–303. <https://doi.org/10.1007/s00442-006-0373-8>
- Pasteels, J. M., Rowell-Rahier, M., Braekman, J. C., & Dupont, A. (1991). Salicin from host plant as precursor of salicylaldehyde in defensive secretion of *Chrysomelinae* larvae. *Physiological Entomology*, 8, 307–314.
- Pickett, J. A., & Khan, Z. R. (2016). Plant volatile-mediated signalling and its application in agriculture: successes and challenges. *New Phytologist*, 212, 856–870.

- R Core Team (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.R-project.org/>
- Rae, A., Pinel, M. P. C., Bastien, C., Sabatti, M., Street, N. R., Tucker, J., ... Taylor, G. (2008). QTL for yield in bioenergy *Populus*: Identifying G x E interactions from growth at three contrasting sites. *Tree Genetics & Genomes*, 4, 97–112.
- Redding-Johanson, A. M., Batth, T. S., Chan, R., Krupa, R., Szmidi, H. L., Adams, P. D., ... Petzold, C. J. (2011). Targeted proteomics for metabolic pathway optimization: Application to terpene production. *Metabolic Engineering*, 13, 194–203. <https://doi.org/10.1016/j.ymben.2010.12.005>
- Rehill, B., Clauss, A., Wieczorek, L., Whitham, T., & Lindroth, R. (2005). Foliar phenolic glycosides from *Populus fremontii*, *Populus angustifolia*, and their hybrids. *Biochemical Systematics and Ecology*, 33, 125–131. <https://doi.org/10.1016/j.bse.2004.06.004>
- Rehill, B., Whitham, T. G., Martinsen, G. D., Schweitzer, J. A., Bailey, J. K., & Lindroth, R. L. (2006). Developmental trajectories in cottonwood phytochemistry. *Journal of Chemical Ecology*, 32, 2269–2285. <https://doi.org/10.1007/s10886-006-9141-9>
- Robinson, K. M., Ingvarsson, P. K., Jansson, S., & Albrechtsen, B. R. (2012). Genetic variation in functional traits influences arthropod community composition in aspen (*Populus tremula* L.). *PLoS ONE*, 7, 1–12.
- Saito, K., Yonekura-Sakakibara, K., Nakabayashi, R., Higashi, Y., Yamazaki, M., Tohge, T., & Fernie, A. R. (2013). The flavonoid biosynthetic pathway in Arabidopsis: Structural and genetic diversity. *Plant Physiology and Biochemistry*, 72, 21–34. <https://doi.org/10.1016/j.plaphy.2013.02.001>
- Schuman, M. C., Allmann, S., & Baldwin, I. T. (2015). Plant defense phenotypes determine the consequences of volatile emission for individuals and neighbors. *eLife*, 4, e04490. <https://doi.org/10.7554/eLife.04490>
- Shuster, S. M., Lonsdorf, E. V., Wimp, G. M., Bailey, J. K., & Whitham, T. G. (2006). Community heritability measures the evolutionary consequences of indirect genetic effects on community structure. *Evolution*, 60, 991–1003. <https://doi.org/10.1111/j.0014-3820.2006.tb01177.x>
- Soetens, P., Pasteels, J. M., Daloze, D., & Kaisin, M. (1998). Host plant influence on the composition of the defensive secretion of *Chrysomela vigintipunctata* larvae (Coleoptera: Chrysomelidae). *Biochemical Systematics and Ecology*, 26, 703–712. [https://doi.org/10.1016/S0305-1978\(98\)00039-8](https://doi.org/10.1016/S0305-1978(98)00039-8)
- Steppuhn, A., Gase, K., Krock, B., Halitschke, R., & Baldwin, I. T. (2004). Nicotine's defensive function in nature. *PLoS Biology*, 2, 1074–1080.
- Stevens, M. L., & Lindroth, R. L. (2005). Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*). *Oecologia*, 145, 298–306.
- Still, W. C., Kahn, M., & Mitra, A. (1978). Rapid chromatographic technique for preparative separations with moderate resolution. *Journal of Organic Chemistry*, 43, 923–925.
- Sundell, D., Mannapperuma, C., Netotea, S., Delhomme, N., Lin, Y.-C., Sjödin, A., ... Street, N. R. (2015). The plant genome integrative explorer resource: PlantGenIE.org. *New Phytologist*, 208, 1149–1156. <https://doi.org/10.1111/nph.13557>
- Taylor, G. (2002). *Populus. Arabidopsis* for forestry. Do we need a model tree? *Annals of Botany*, 90, 681–689. <https://doi.org/10.1093/aob/mcf255>
- Tahvanainen, J., Niemela, P., & Henttonen, H. (1991). Chemical aspects of herbivory in boreal forest-feeding by small rodents, hares and cervids. In P. T. Palo, & C. T. Robbins (Eds.), *Plant defenses against mammalian herbivory* (pp. 115–131). Boca Raton, MA: CRC Press.
- Touominen, L. K., Johnson, V. E., & Tsai, C. J. (2011). Differential phylogenetic expansions in BAHD acyltransferases across five angiosperm taxa and evidence of divergent expression among *Populus* paralogues. *BMC Genomics*, 12, 236. <https://doi.org/10.1186/1471-2164-12-236>
- Tsai, C.-J., Harding, S. A., Tschaplinski, T. J., Lindroth, R. L., & Yuan, Y. (2006). Genome-wide analysis of the structural genes regulating defense phenylpropanoid metabolism in *Populus*. *New Phytologist*, 172, 47–62. <https://doi.org/10.1111/j.1469-8137.2006.01798.x>
- Tsai, C.-J., Kayal, W. E., & Harding, S. A. (2006). *Populus*, the new model system for investigating phenylpropanoid complexity. *International Journal of Applied Science and Engineering*, 4, 221–233.
- Turtola, S., Rousi, M., Puseenius, J., Yamaji, K., Heiska, S., Tirkkonen, V., ... Julkunen, Tiitto R. (2005). Clone-specific responses in leaf phenolic glycosides of willows exposed to enhanced UVB radiation and drought stress. *Global Change Biology*, 11, 1655–1663. <https://doi.org/10.1111/j.1365-2486.005.01013.x>
- Tuskan, G. A., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., ... Schein, J. (2006). The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science*, 313, 1596–1604. <https://doi.org/10.1126/science.1128691>
- van Ooijen, J. (1999). LOD significance thresholds for QTL analysis in experimental populations of diploid species. *Heredity*, 83, 613–624. <https://doi.org/10.1038/sj.hdy.6886230>
- Waltz, A. M., & Whitham, T. G. (1997). Plant development affects arthropod communities: Opposing impacts of species removal. *Ecology*, 78, 2133–2144. [https://doi.org/10.1890/0012-9658\(1997\)078\[2133:PDAACO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[2133:PDAACO]2.0.CO;2)
- Wang, S., Basten, C. J., & Zeng, Z.-B. (2012). *Windows QTL Cartographer 2.5*. Department of Statistics, North Carolina State University, Raleigh, NC. Retrieved from <http://statgen.ncsu.edu/qtlcart/WQTLcart.htm>
- Warren, J. M., Bassman, J. H., Fellman, J. K., Mattinson, D. S., & Eigenbrode, S. (2003). Ultraviolet-B radiation alters phenolic salicylate and flavonoid composition of *Populus trichocarpa* leaves. *Tree Physiology*, 23, 527–535. <https://doi.org/10.1093/treephys/23.8.527>
- Wegrzyn, J. L., Eckert, A. J., Choi, M., Lee, J. M., Stanton, B. J., Sykes, R., ... Neale, D. B. (2010). Association genetics of traits controlling lignin and cellulose biosynthesis in black cottonwood (*Populus trichocarpa*, Salicaceae) secondary xylem. *New Phytologist*, 188, 515–532. <https://doi.org/10.1111/j.1469-8137.2010.03415.x>
- Wessa, P. (2016). *Box-Cox linearity plot (v1.0.4)* in *Free Statistics Software (v1.1.23-r6)*. Office for research development and education. Retrieved from http://www.wessa.net/rwasp_boxcoxlin.wasp/
- Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., ... Fischer, D. G. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nature Reviews Genetics*, 7, 510–523. <https://doi.org/10.1038/nrg1877>
- Whitham, T. G., DiFazio, S. P., Schweitzer, J. A., Shuster, S. M., Allan, G. J., Bailey, J. K., & Woolbright, S. A. (2008). Extending genomics to natural communities and ecosystems. *Science*, 320, 492–495. <https://doi.org/10.1126/science.1153918>
- Whitham, T. G., Martinsen, G. D., Floate, K. D., Dungey, H. S., Potts, B. M., & Keim, P. (1999). Plant hybrid zones affect biodiversity: Tools for a genetic-based understanding of community structure. *Ecology*, 80, 416–428. [https://doi.org/10.1890/0012-9658\(1999\)080\[0416:PHZABT\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[0416:PHZABT]2.0.CO;2)
- Wickham, H. (2009). *ggplot2: Elegant graphics for data analysis*. New York, NY: Springer. <https://doi.org/10.1007/978-0-387-98141-3>
- Woolbright, S. A., DiFazio, S. P., Yin, T., Martinsen, G. D., Zhang, X., Allan, G. J., ... Keim, P. (2008). A dense linkage map of hybrid cottonwood (*Populus fremontii* x *P. angustifolia*) contributes to long-term ecological research and comparison mapping in a model forest tree. *Heredity*, 100, 59–70. <https://doi.org/10.1038/sj.hdy.6801063>
- Wooley, S. C., Walker, S., Vernon, J., & Lindroth, R. L. (2008). Aspen decline, aspen chemistry, and elk herbivory: Are they

linked? *Rangelands*, 30, 17–21. [https://doi.org/10.2111/1551-501X\(2008\)30\[17:ADACAE\]2.0.CO;2](https://doi.org/10.2111/1551-501X(2008)30[17:ADACAE]2.0.CO;2)

Wulschleger, S. D., Jansson, S., & Taylor, G. (2002). Genomics and forest biology: *Populus* emerges as the new perennial favorite. *Plant Cell*, 14, 2651–2655. <https://doi.org/10.1105/tpc.141120>

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Woolbright SA, Rehill BJ, Lindroth RL, et al. Large effect quantitative trait loci for salicinoid phenolic glycosides in *Populus*: Implications for gene discovery. *Ecol Evol.* 2018;8:3726–3737. <https://doi.org/10.1002/ece3.3932>