

GENOME-WIDE ASSOCIATION STUDY REVEALS GENES ASSOCIATED WITH MITE  
RECRUITMENT PHENOTYPES IN THE DOMESTICATED GRAPEVINE (*VITIS*  
*VINIFERA*)

By

Erika R. LaPlante

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## ABSTRACT

### GENOME-WIDE ASSOCIATION STUDY REVEALS GENES ASSOCIATED WITH MITE RECRUITMENT PHENOTYPES IN THE DOMESTICATED GRAPEVINE (*VITIS VINIFERA*)

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Plants in the grape genus *Vitis* have varying densities of trichomes and mite-domatia on their leaves, which facilitate the recruitment, retention, and reproduction of beneficial mites. By increasing the abundance of mites on grape leaves, these phenotypes promote a defense mutualism contributing to the control of grape pests and pathogens. Identification of the genes controlling these phenotypes would inform our understanding of the genetics underlying mite-plant mutualistic interactions and could lead to breeding domesticated *Vitis vinifera* L. varieties that are naturally defended against pathogens. Little is known about the genetics underlying mite recruitment phenotypes in *Vitis*. We conducted a GWAS to determine the genetic architecture of mite recruitment traits in *V. vinifera* using 399 cultivars from a common garden diversity panel. We investigated eight traits previously established in the literature associated with an increase in beneficial mite abundance. We found single nucleotide polymorphisms (SNPs) significantly associated with each mite recruitment trait investigated. Corresponding gene annotations of SNP genetic coordinates revealed notable gene associations, including a trichome development gene, and a physiological defense response gene, suggesting these genetic regions may have a large impact on mediating mite-plant interactions in this species. Our findings are among the first to investigate which genes underly ecologically important mutualisms between plants and beneficial mites and suggest promising candidate genes for breeding and genetic editing to increase naturally occurring predator-based defenses in grapes.

This is dedicated to my family: Shane, Shelley, & Kari.  
Thank you for always supporting me and my ambitions.

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## INTRODUCTION

Plants have evolved a wide variety of novel traits to defend themselves against enemies. A strategy of “indirect defense” has evolved multiple times, in which plants attract and retain beneficial arthropods (such as predators or microbivores) in return for protection against herbivores and pathogens, facilitating defense through tri-trophic interactions (Weber et al., 2012 & Bronstein, Alarcón, & Geber, 2006). In these interactions, plants can provide rewards to beneficial arthropods (either nutrition or shelter) or signal the presence of prey to predators (using volatile organic compounds) (Quintero, Barton, and Boege, 2013; Fatouros et al., 2008; De Moraes et al., 1998). Though the benefit of indirect defense traits is well recognized ecologically, for most traits we know little about their underlying genetic drivers (Mooney and Agrawal, 2008 & Weber et al., 2012). Determining the genetic control of indirect defensive traits will give insight into the underlying causes and constraints of defense strategies across the tree of life. Furthermore, identifying genes responsible for indirect defense can potentially aid in agricultural development, facilitating the breeding or genetic engineering of crops with indirect defenses. Here, we investigate the genomic underpinnings of indirect defense using an ecologically well studied, but genetically under-explored, system: traits that facilitate interactions between plants and “bodyguard” mites that consume plant pests (herbivores and pathogenic microbes).

Several morphological plant traits have been well demonstrated to attract and retain mutualistic mites (Pemberton and Turner, 1989). Trichomes on the lamina and raised, hairy veins are associated with increased abundance of beneficial mites on leaves in some species (Hanley et al., 2007 & Loughner et al., 2008). Loughner et al. (2008) found a positive non-linear relationship between trichome abundance on leaves of 12 different *Vitis* varieties and the

abundance of predatory mite, *Typhlodromus pyri* (Scheuten). Furthermore, experiments have shown that an increase in trichomes concentrated in the vein axils leads to an increase in beneficial mite abundance, which is associated with a decrease in the abundance of pathogens that attack *Vitis* (O'Dowd and Willson, 1997; Norton et al., 2000; English-Loeb et al., 2002, 2005, 2007; Melidossian et al., 2005; Duso et al., 2009). Together, by recruiting and retaining mutualistic mites on leaves, leaf and vein trichomes facilitate mites as an important biocontrol in apples, grapes, and other woody plants that exhibit these morphological traits.

Aside from the more general trichome traits described above, some plants also possess more specialized structures for mite recruitment and retention. Most notable are leaf domatia (hereafter domatia), small structures on plant leaves that provide shelter for beneficial mites. Domatia consist of depressions in the leaf surface, covered with either a dense layer of trichomes or a fold of tissue, located in the abaxial vein axils (where one vein meets another on the underside of the leaf). Domatia are well documented to mediate mutualistic interactions between plants and mite bodyguards. Mites thrive in the hospitable environment that domatia provide on the leaf surface, and in turn, mites eat pests (herbivores and pathogenic microbes) on the plant leaves (O'Dowd and Willson, 1997; Norton et al., 2000; English-Loeb et al., 2002, 2005, 2007; Melidossian et al., 2005; Duso et al., 2009). Domatia can increase plant fitness by increasing beneficial mite abundance, which decreases the abundance of pests and pathogens on leaves (Agrawal, Karban, & Colfer 2000; Grostal & O'Dowd 1994; & Norton et al., 2000). Domatia are found on thousands of woody plant species including black pepper, oak, cherry, *Viburnum*, coffee, grape, and avocado (Brouwer and Clifford, 1990). A series of regional surveys of woody perennial plants found a positive association between the presence of domatia and the abundance of carnivorous and fungivorous mites (O'Dowd & Willson, 1989; Pemberton & Turner, 1989;

Brouwer & Clifford, 1990; Willson, 1991; O'Dowd & Pemberton, 1994; Rozario, 1995; Walter & O'Dowd, 1995b; O'Dowd & Pemberton, 1998), pointing to domatia as a common phenotype mediating a widespread mutualistic indirect defense interaction.

Together, a large body of research supports trichome and domatia phenotypes as facilitating beneficial mite populations on plant leaves, making them strong candidates for work investigating the underlying genetic drivers of indirect defensive traits. However, little work has investigated the genetic underpinnings of these traits in the context of mite-defense. Only one study has investigated the genetics of domatia and mite related trichome phenotypes to date (Barba et al, 2019). In this study, the authors used a Quantitative Trait Loci (QTL) approach, investigating a segregating F1 family derived from the cross of two *Vitis* hybrids to reveal QTL associated with leaf trichome and domatia traits as well as predatory mite abundance (Barba et al., 2019). To our knowledge, this is the only study to date that has examined the genetic underpinnings of domatia, despite their ecological importance across plants. However, although not directly linked to mite abundance, previous research on trichome phenotypes more generally has provided a strong foundation of work linking trichome traits to potential genetic loci. For example, studies have identified genes contributing to the triggering of trichome differentiation in *Arabidopsis thaliana* (L.) Heynh (Balkunde, Pesch, and Hülkamp, 2010), determined the genetics of developmental variation and phenotypic plasticity in trichome density in *Erythranthe [Mimulus] guttatus* (Holeski, Chase-Alone, and Kelly, 2010), and determined the genetics involved in the formation of epidermal cells and trichomes in tomatoes (Zhang et al., 2015). Though these studies provide insight into the genetics of trichome development more generally, they do not link trichome-related genes to indirect defense/mutualisms, as they were not investigated in a mite-plant system.

Here, we investigate the genetic control of mite-defense related trichome and domatia traits in the cultivated grape, *Vitis vinifera*, a particularly promising system for linking mite defense phenotypes to genotypes. A large body of work supports leaf domatia and leaf trichome phenotypes as traits that increase the abundance of beneficial mites on the leaves of grape plants (Barba et al., 2019; English-Loeb et al., 2002, 2005, 2007 & Norton et al., 2000). Domatia and leaf trichome phenotypes increase the abundance of mycophagous (family *Tydeidae*) and predacious mites (family *Phytoseiidae*) on *Vitis* leaves (Karban et al., 1995). These increases in mite abundances are associated with the biological control of grape powdery mildew, *Uncinula necator* (British Columbia: Ministry of Agriculture, 2015) and decreased abundance of harmful spider mites (family *Tetranychidae*) across *Vitis* species (Karban et al., 1995). This interaction is agriculturally important, as powdery mildew and spider mites are both common and widespread pests that are routinely controlled by spraying vineyards with sulfur (British Columbia: Ministry of Agriculture, 2015).

In tandem with their role in mediating indirect defense, trichome and domatia phenotypes in *Vitis* are also heritable (English-Loeb et al., 2002 & 2005), and the growing genomic toolkit available for *Vitis* make the genetic investigation of mite indirect defense phenotypes in *Vitis* highly feasible. However, only one study (the QTL study of Barba et al. mentioned above) has investigated the underlying genetic mechanisms of mite-related traits in *Vitis*. Further, while the Barba et al. QTL study informed our understanding of the underlying genetic basis of indirect defense in *Vitis*, the associated QTLs were limited to the allelic diversity that segregates between the parents of the F1 cross and the amount of recombination that occurred during the creation of the Recombinant Inbred Lines (RILs), placing a limit on the mapping resolution. Additional work in the form of an association mapping panel could help narrow the position of the locus and

further reduce the list of candidate genes. In contrast to a QTL, a Genome Wide Association study (GWAS) can offer valuable insight into the number of loci that contribute to the genotype and their respective contribution to the phenotype (Korte and Farlow, 2013). In a sufficiently large panel/population, GWAS records more recombination events that contribute to a higher resolution of intra- and inter-specific variation, increasing the opportunity for identifying regions associated with leaf phenotypes across a more diverse panel of cultivars.

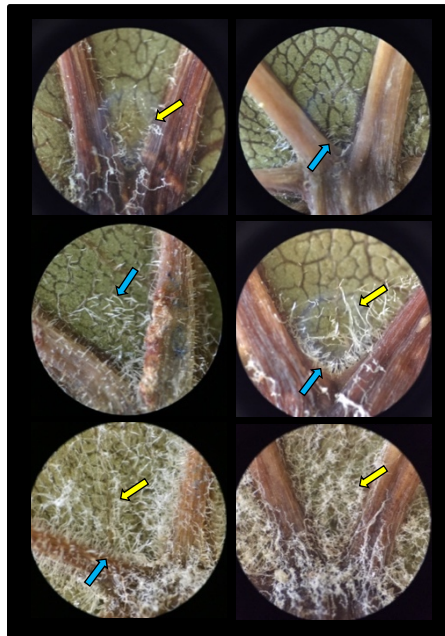
To fill the gap in the literature on the underlying genetic drivers of indirect defense traits in the context of mite-defense, we performed a GWAS of beneficial-mite related phenotypes using the cultivated grapevine, *V. vinifera*. We investigated the genetic associations of leaf domatia and mite-related leaf trichome phenotypes across a panel of 399 cultivars of domesticated grapevine. We capitalize on the large amount of phenotypic variation in *V. vinifera* for mite-related phenotypes, the large body of ecological research tying these phenotypes to mite defense, and a pre-established diversity panel common garden of *V. vinifera* to identify single-nucleotide polymorphisms (hereafter, SNPs) associated with mite indirect defense. Our goal is to provide candidate genetic regions underlying key phenotypes for the recruitment and retention of beneficial mites on grape leaves, and to provide insight into the genetic control of mite-plant defense mutualisms more generally.



## METHODS

### *Study system*

The domesticated grapevine *V. vinifera* is a perennial vine in the family Vitaceae. Archeological records propose *V. vinifera* subsp. *vinifera* (hereafter *V. vinifera*) was first domesticated about 6,000-8,000 years ago in the Near East from its wild progenitor, *Vitis vinifera* subsp. *sylvestris* (McGovern, 2019). Domesticated grapevines have been cultivated primarily via cuttings since this time, resulting in cultivars that have changed slowly or not at all (Adam-Blondon et al., 2016). Most current cultivars of grapevines are hermaphroditic and are highly heterozygous, which require vegetative propagation to maintain the phenotypic features that have driven the grapevine industry for centuries. Therefore, grapevine cultivars represent highly conserved gene combinations due to the maintenance of the most desirable genetic structures (Adam-Blondon et al., 2016). In grapevines, most agriculturally important traits do not have a simple genetic basis, but rather are controlled by large numbers of genes with minor effects (Adam-Blondon et al., 2016). The recent completion of the sequenced grapevine genome (*V. vinifera*: 487-500Mbp genome; 2n=38 chromosomes) and an increase in construction of genetic maps for quantitative trait analysis has accelerated the development of grapevine genomics and is generating an explosion in grapevine research (Jaillon et al., 2007). *Vitis vinifera* has considerable variation in mite-recruitment phenotypes (English-Loeb et al., 2002), including domatia presence/absence, domatia size, and leaf trichome density, in part due to targeted breeding with wild species such as *V. riparia*, a wild species from the Eastern North America with large domatia (Reisch & Pratt, 1996), making it a promising system for a GWAS targeted at mite defense phenotypes (Figure 1).



**Figure 1. Trichome diversity in *Vitis vinifera*.** Photos taken through stereomicroscope lens of trichomes immediately surrounding left midvein axil at base of leaf on abaxial side. Yellow arrows point to prostrate hairs. Blue arrows point to erect hairs.

#### ***Association panels: genetic relationship***

The plant material used in our analysis were collected from a common garden grapevine diversity panel of  $n = 399$  cultivars of *V. vinifera* that originated from diverse geographical regions. This diversity panel is maintained at the Wolfskill Farms in Winters, CA and is one of two Plant Genetics Repository Units (PGRU) maintained by the United States Department of Agriculture, Agricultural Research Service (USDA-ARS). The genetic dataset was originally developed by Myles et al. (2011) as part of an effort to characterize the USDA-ARS germplasm collection on a genome-wide scale. The data consist of an Illumina GA sequenced 9kSNP array. Myles et al. (2011) determined the linkage disequilibrium (LD), as measured by  $r^2$  between SNPs, decays rapidly in this dataset. Their analyses of haplotype diversity and LD suggested

grape domestication went through a weak bottleneck and therefore present-day cultivars maintain a large proportion of the haplotype diversity observed in the ancestral *V. sylvestris*, while the decay of LD appears unchanged between *V. vinifera* and *V. sylvestris*. Furthermore, their analysis concluded there is extensive clonal relationships among cultivars and a complex pedigree structure within *V. vinifera* due to vegetative propagation practiced in viticulture.

### ***Phenotyping***

To quantify phenotypic variation in mite-related traits across the panel, we collected three mid-shoot, fully expanded adult leaves from each of the 399 individual plants (~1,200 total leaves) in June of 2018. All leaves used in this study occurred beyond the sixth node from the base of the shoot coming from wood of the preceding year (Galet, 1979).

We measured a total of eight leaf traits on all collected leaves (Table 1). All leaf trait measurements were taken using a stereomicroscope (12X) and an ocular micrometer (Figure 2). We measured two leaf traits on fresh leaves, domatia cave depth and midvein depth, before the leaves were pressed and dried for transport back to Michigan State University. Domatia cave depth was measured by placing a flat head sewing pin, flat side down into a select domatia (located in the vein axil at the base of the midvein, closest to the petiole on the abaxial side of the leaf) and marking the pin with a fine tip sharpie at the height of the midvein. The length from the base of the pin to the sharpie mark was measured using an ocular micrometer and recorded. Midvein depth was measured using the same methods, but halfway between the base of the midvein and the next vein axil.

We measured the remaining six traits on pressed leaves from the fall of 2018 to the fall of 2019. These three domatia size measurements (total domatia size index, erect hair domatia size

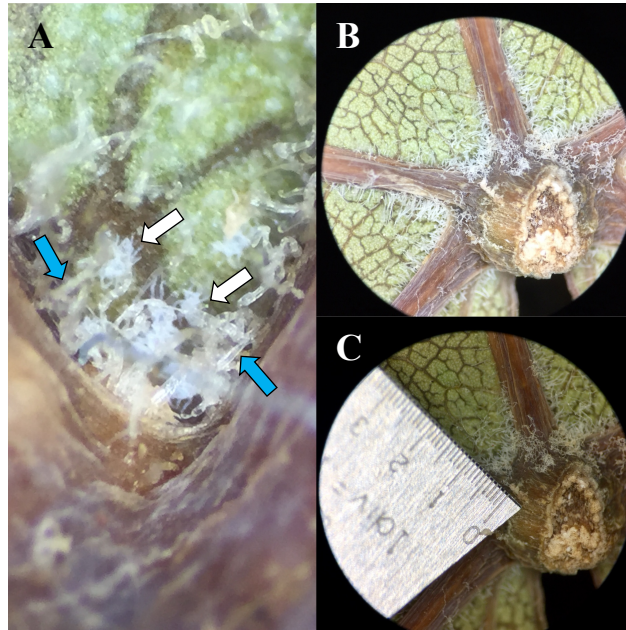
index, and prostrate hair domatia size index), as well as three laminar leaf trichome traits (presence/absence of abaxial leaf trichomes, presence/absence of erect hair on abaxial leaf surface, presence/absence of prostrate hair on abaxial leaf surface). *Vitis vinifera* species have two types of trichomes, erect and prostrate, that vary among grapevine cultivars in their relative abundance and spatial density. Erect trichomes are conical, approximately 300 µm long, and prostrate trichomes are cylindrical, approximately 2000 µm long (becoming senescent and flattened as leaves age) (Gago et al., 2016). When the erect and prostrate trichomes become entangled on the adaxial and abaxial leaf surface, they form a ‘hair coat’ that is supported by the erect trichomes and can be approximately 1.5 times thicker than the leaf lamina (Gago et al., 2016). The two types of non-glandular trichomes are not only found across the leaf surface but can also contribute to the structure of domatia. Domatia size index, a trait commonly used in ecological studies of mite-plant interactions, measures the combined density and diameter of erect hairs and prostrate hairs that make up the domatia covering. Likewise, presence/absence of total abaxial leaf hairiness includes the measurements of the presence/absence of erect hair in full abaxial leaf hairiness and the presence/absence of prostrate hair in full abaxial leaf hairiness. Domatia size index, erect hair domatia size index, and prostrate hair domatia size index leaf hair measurements were defined and measured in accordance to the Descriptors for Grapevine (IPGRI et al., 1997), a standard rating system used by grape breeders. These leaf traits were scored using OIV and UPOV codes for the respective traits (O-084, U-33 and O-085, U-34) on a rating index: 0 = Absent, 1 = Very sparse, 3 = Sparse, 5 = Medium, 7 = Dense, 9 = Very dense. Domatia size index was calculated by multiplying domatia diameter (as measured with the ocular micrometer from the crux of the vein axil out towards the point where the trichome density significantly decreased) by the domatia rating index for each domatium (English-Loeb et al.,

2002). Domatia depth, midvein depth, domatia size index, erect hair domatia size index, and prostrate hair domatia size index were measured as continuous traits, while the presence/absence of total abaxial leaf hairiness, presence/absence of erect hair in full abaxial leaf hairiness, and presence/absence of prostrate hair in full abaxial leaf hairiness were all measured as discrete traits. Average cultivar trait values (continuous and discrete) were calculated as the mean of the three replicate leaves per cultivar for each trait. There were no occurrences where discrete trait measurements differed amongst replicates of the same cultivar.

We tested for correlations between phenotypes using Pearson's correlation matrix and "holm" corrected p-values (Holm, 1979) for multiple comparisons using the `corr.test` function in the `psych` package (Revelle, 2019). We visualized the correlation matrix results using the `corrplot.mixed` function in the R package `corrplot` (Wei and Simko, 2017). All analyses were run using R version 3.6.3 (R Core Team, 2013).

**Table 1. List of the eight phenotypes and the respective shortened descriptors and abbreviations used.** For trait, presence/absence of abaxial leaf trichomes, the shortened phenotype of full leaf glab/pub was used (glab: *glabrous* indicates no hair on the abaxial leaf surface and pub: *pubescent* indicates a hairy abaxial leaf surface) and the abbreviated phenotype reflects this. For trait, presence/absence of erect hair on abaxial leaf surface, the shortened phenotype of full leaf erect was used and the abbreviated phenotype reflects this. For trait, presence/absence of prostrate hair on abaxial leaf surface, the shortened phenotype of full leaf prostrate was used and the abbreviated phenotype reflects this.

| <b>Descriptive Phenotype</b>                               | <b>Shortened Phenotype</b> | <b>Abbreviated Phenotype</b> |
|------------------------------------------------------------|----------------------------|------------------------------|
| Cave depth                                                 | Cave depth                 | CD                           |
| Midvein depth                                              | Midvein depth              | MD                           |
| Domatia size index                                         | Domatia size               | DS                           |
| Erect hair domatia size index                              | Erect domatia size         | EDS                          |
| Prostrate hair domatia size index                          | Prostrate domatia size     | PDS                          |
| Presence/absence of abaxial leaf trichomes                 | Full leaf glab/pub         | FLGP                         |
| Presence/absence of erect hair on abaxial leaf surface     | Full leaf erect            | FLE                          |
| Presence/absence of prostrate hair on abaxial leaf surface | Full leaf prostrate        | FLP                          |



**Figure 2. Visualization of mites in domatia and how domatia diameter was measured.**

Domatia at the base of the midvein on *Vitis vinifera* cultivars (image B and C are from the same leaf; image A is from a different leaf and cultivar). **(A)** Mite exoskeletons in domatium.

White arrow indicates mite exoskeletons and blue arrow indicates trichomes. **(B)** View of domatia under the stereomicroscope. **(C)** View of domatia under the stereomicroscope, with the ocular micrometer used to measure domatia diameter.

### ***Genome-wide association study analysis***

We conducted a GWAS analysis for each trait independently using the GAPIT R package (Version 1, Zhang et al., 2010). To avoid rare variants contributing to false positives, we used the qqnorm statistical method (Becker, Chambers, and Wilks, 1988) to conduct normality transformations of specific phenotypic traits before inputting the phenotypic data into GAPIT. To account for the population structure in our analysis, we used GAPIT's built in Bayesian information criterion (BIC)-based model selection to find the optimal number of Principle Components for inclusion in the GWAS models (capped at 3 PCs). To visualize population structure, we plotted a kinship matrix calculated as the proportion of shared alleles between cultivars. We calculated the kinship matrix using the VanRaden method (VanRaden, 2008) and performed GWAS with the optimum compression level using the default clustering algorithm (average) and group kinship type (mean). To account for non-independence in the data, we used a mixed linear model (MLM), which allowed for both fixed and random effects (Yu et al., 2006). We performed the GWAS analysis using Mixed Linear Model (MLM) and a Compressed Mixed Linear Model (CMLM (Zhang et al., 2010)) for continuous and discrete traits, respectively. We used a Minor Allele Frequency (MAF)  $\geq 5\%$  for continuous and discrete traits. Linkage Disequilibrium (LD) in *V. vinifera* was previously characterized by Myles et al. (2010) and is generally low ( $r^2 < 0.2$ ) but remains above background levels to  $\sim 10\text{Kb}$ . To assesses how well the model used in the GWAS accounts for population structure and familial relatedness we produced quantile-quantile (QQ) –plots for all analyses. All analyses were run using R version 3.6.3 (R Core Team, 2013), and all scripts were deposited in a data dryad repository.



### ***Identification of Single-Nucleotide Polymorphisms and Candidate Genes***

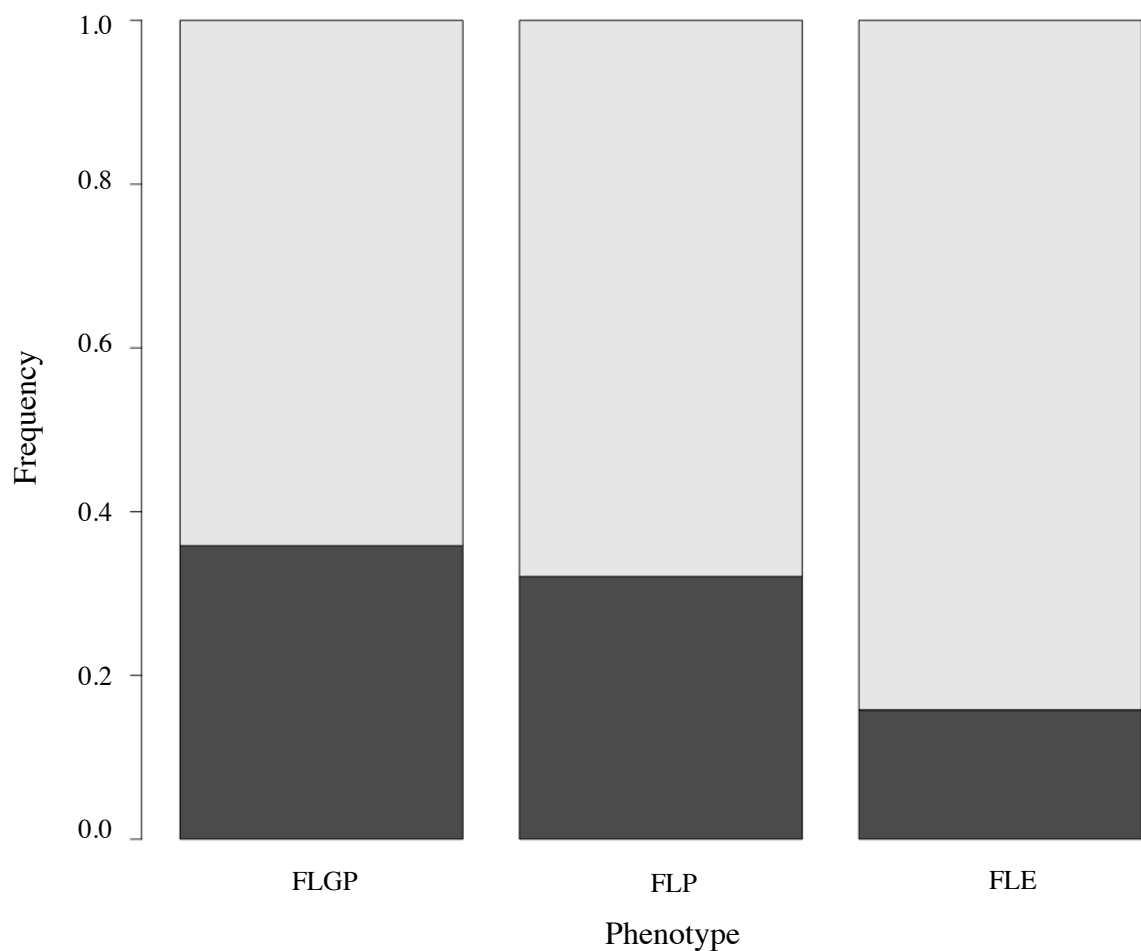
Significant cutoff thresholds affect candidate gene identification in GWAS. Controlling for false positives is crucial, however, the effect of false negatives should not be overlooked. False negatives can occur if the cutoff value is too stringent (a common caveat to using a Bonferroni correction threshold). To identify SNPs significantly associated with our traits of interest, we used a bootstrapping method developed by Mamidi et al. (2014). Significant SNPs in the GWAS were determined using two cutoff thresholds based on the empirical distribution of the P-values after 100 bootstraps: (i) a stringent cutoff where SNPs fall in the lower 0.01 percentile tail and (ii) a relaxed cutoff where SNPs fall in the 0.1 percentile tail. The more stringent cutoff was comparable to the Bonferroni alpha threshold (Bonferroni, 1936).

To identify genes of interest, we compared significant SNPs to the *V. vinifera* *Genoscope.12X* (Jaillon et al., 2007). Candidate genes were identified by searching a 150 Kb upstream and downstream window around each significant SNP (total window searched 300 Kb). For each candidate gene, we report the associated *Arabidopsis* annotated genes in Supplementary Table 1-8. We matched the *Arabidopsis* annotations for each gene association using the *Genoscope.12X* annotation information files in the JGI Genome Portal in Phytozome v12.1 (Jaillon et al., 2007).

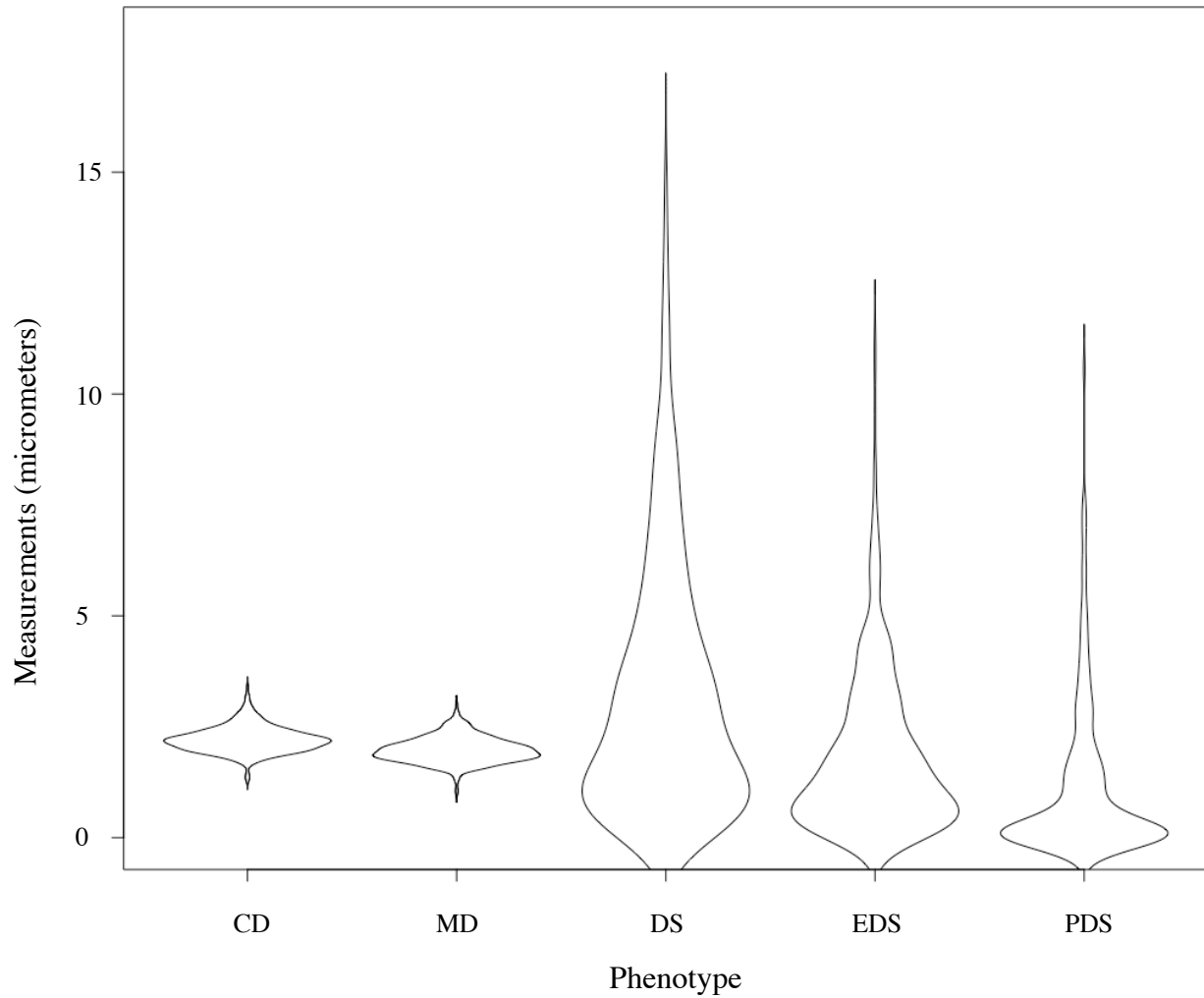
## RESULTS

### *Phenotyping*

We found considerable variation in mite-recruitment phenotypes across our common garden genotypes. Of the 399 cultivars, 256 cultivars were glabrous (absence of trichomes) on the full abaxial leaf surface, and 143 cultivars were pubescent (trichomes present) on the full abaxial leaf surface (Figure 3). Of the abaxial full leaf pubescent cultivars, 15 had only erect hairs (of which 13 had erect and prostrate domatia and the remaining two only had erect domatia), 80 had only prostrate hairs (of which 76 had erect and prostrate domatia, two had only prostrate domatia, and 2 had only erect domatia), and 48 had both erect and prostrate hairs. Domatia size index measurements ranged from 0.00-15.00 $\mu\text{m}$  (mean 3.27) (Figure 4) and was positively correlated with both erect domatia size index (range 0.00-11.25 $\mu\text{m}$ , mean 1.86,  $r = 0.599$ , Figure 5) and prostrate domatia size index (range 0.00-10.60 $\mu\text{m}$ , mean 1.18,  $r = 0.690$ , Table 2 and Figure 5). Cave depth measurements ranged from 1.30-3.40 micrometers ( $\mu\text{m}$ ) (average 2.21) and midvein depth measurements ranged from 1.00-3.00 $\mu\text{m}$  (average 1.98), indicating that domatia depth was larger than midvein depth on average (Figure 4). Cave depth and midvein depth were positively correlated ( $r = 0.695$ , Table 2 and Figure 5).



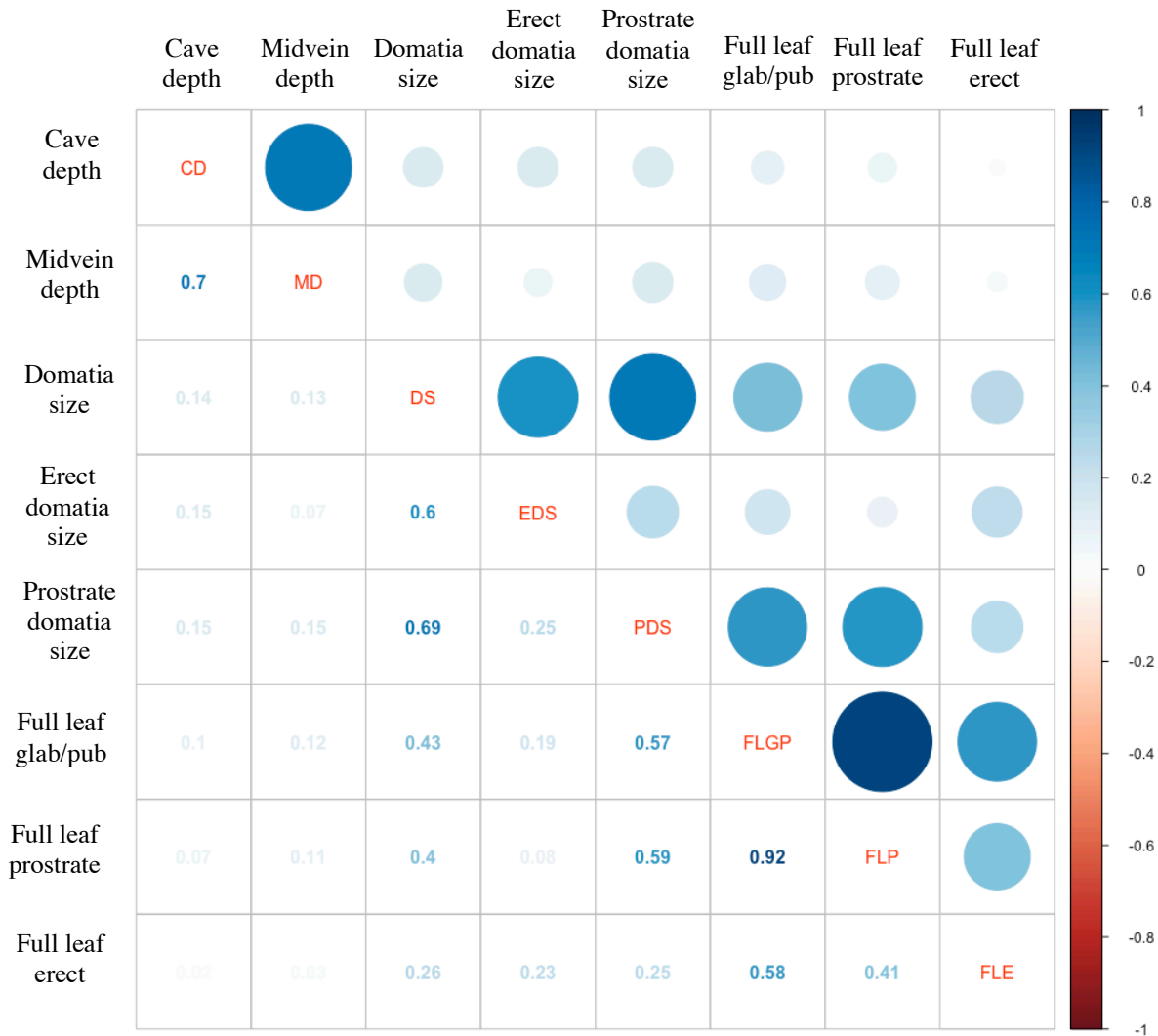
**Figure 3. Greater frequency of hairs present for traits measured as the presence or absence of hairs on abaxial leaf surface.** The stacked boxplot of phenotypes measured with discrete data. Presence of trait (top) in light grey. Absence of trait (bottom) in dark grey. Phenotypes: full leaf glab/pub (FLGP), full leaf erect (FLE), and full leaf prostrate (FLP).



**Figure 4. Violin plots illustrating the density distribution of measurements for each phenotype.** The width of each violin plot represents the proportion of the data in that interval. CD and MD are measurements of depth (units: micrometers). DS, EDS, and PDS are measurements of size index (domatia size index = diameter of domatia \* density rating; units: micrometers). Abbreviations: cave depth (CD), midvein depth (MD), domatia size (DS), erect domatia size (EDS), prostrate domatia size (PDS).

**Table 2. Correlation coefficients (r) between each phenotype.** Numerical data underlying results associated with the correlation matrix in Figure 5. Correlation coefficients are on the top half of the matrix, and p-values are on the bottom half of the matrix.

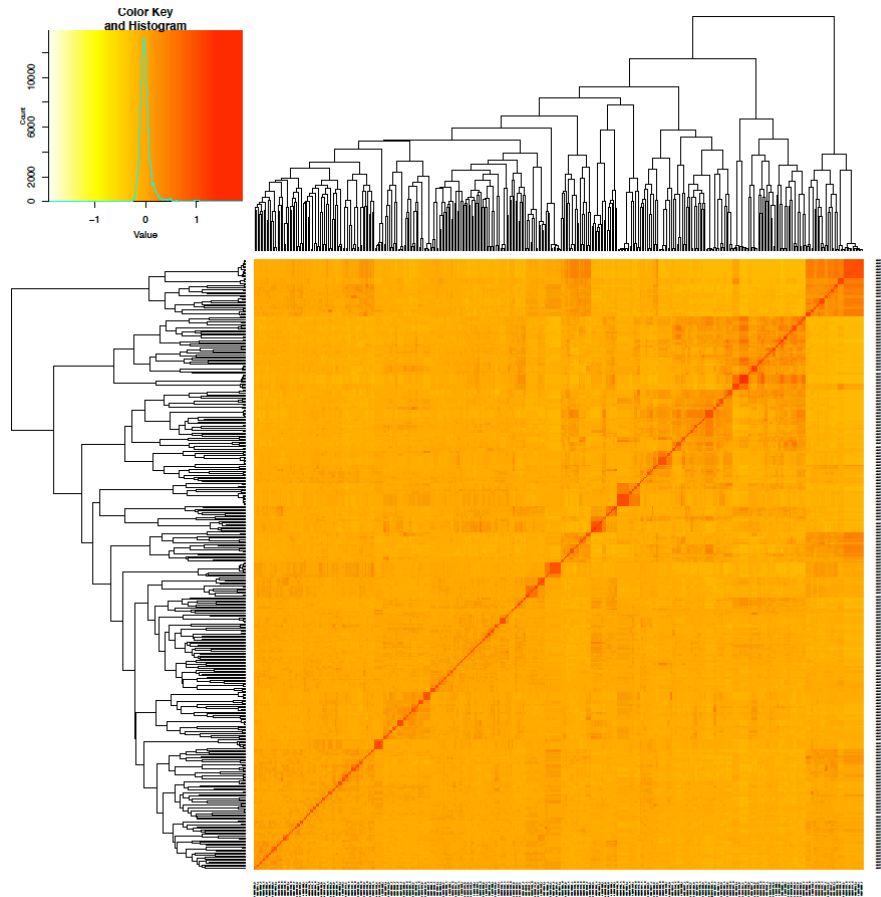
|                               | <b>Cave depth</b> | <b>Midvein depth</b> | <b>Domatia size</b> | <b>Erect domatia size</b> | <b>Prostrate domatia size</b> | <b>Full leaf glab/pub</b> | <b>Full leaf prostrate</b> | <b>Full leaf erect</b> |
|-------------------------------|-------------------|----------------------|---------------------|---------------------------|-------------------------------|---------------------------|----------------------------|------------------------|
| <b>Cave depth</b>             | NA                | 0.695                | 0.143               | 0.148                     | 0.148                         | 0.096                     | 0.072                      | 0.020                  |
| <b>Midvein depth</b>          | <0.001            | NA                   | 0.130               | 0.070                     | 0.149                         | 0.120                     | 0.106                      | 0.033                  |
| <b>Domatia size</b>           | 0.041             | 0.082                | NA                  | 0.599                     | 0.690                         | 0.428                     | 0.404                      | 0.257                  |
| <b>Erect domatia size</b>     | 0.037             | 0.605                | <0.001              | NA                        | 0.247                         | 0.185                     | 0.082                      | 0.233                  |
| <b>Prostrate domatia size</b> | 0.037             | 0.037                | <0.001              | <0.001                    | NA                            | 0.575                     | 0.587                      | 0.247                  |
| <b>Full leaf glab/pub</b>     | 0.336             | 0.129                | <0.001              | 0.003                     | <0.001                        | NA                        | 0.920                      | 0.579                  |
| <b>Full leaf prostrate</b>    | 0.605             | 0.239                | <0.001              | 0.511                     | <0.001                        | <0.001                    | NA                         | 0.409                  |
| <b>Full leaf erect</b>        | 1.000             | 1.000                | <0.001              | <0.001                    | <0.001                        | <0.001                    | <0.001                     | NA                     |



**Figure 5. The graphical display of a correlation matrix, confidence interval, for all 8 phenotypes measured.** Positive correlations are displayed in blue and negative correlations in red. Color intensity and the size of the circle are proportional to the correlation coefficients. Abbreviations: cave depth (CD), midvein depth (MD), domatia size (DS), erect domatia size (EDS), prostrate domatia size (PDS), full leaf glab/pub (FLGP), full leaf erect (FLE), full leaf prostrate (FLP).

### ***Genome-wide association study analysis***

Genome-wide association analyses in GAPIT revealed significant associations for each mite related phenotype and SNPs in the *V. vinifera* panel. Kinship matrices confirmed findings of Myles et al. (2011), revealing low population structure across the panel (Figure 6). We found the best fit model was the Mixed Linear Model (MLM). We ran this MLM with zero PCs across all traits due to BIC-model selection results which was consistent with the lack of population structure found in the kinship matrix. The quantile-quantile (QQ) –plots (Figure 7.1B & 7.2B) reflected a tight correlation between the negative logarithms of the P-values from the models fitted in GWAS and the expected value under the null hypothesis, which confirms that the model is accounting for the kinship matrix.

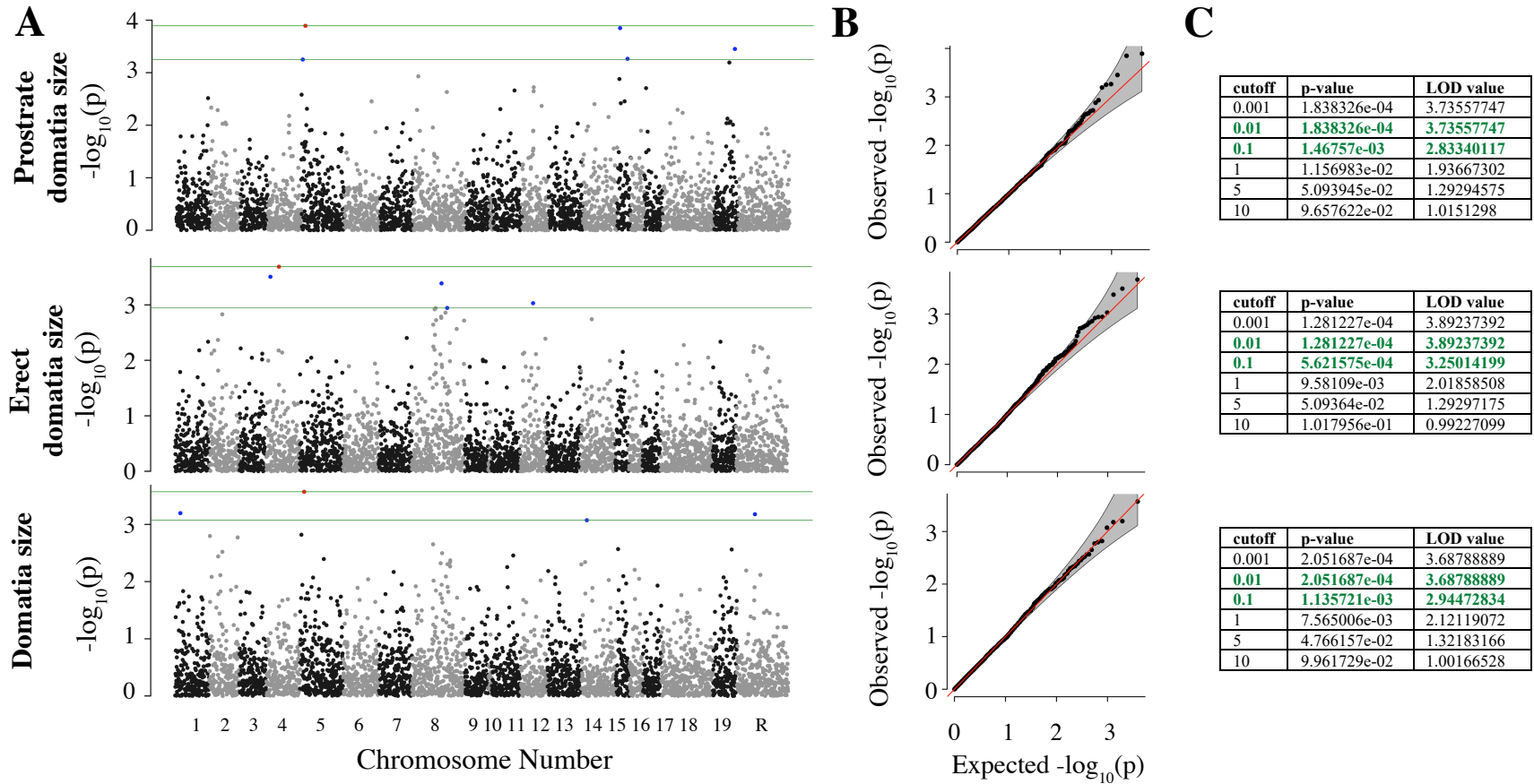


**Figure 6. Kinship matrix shows low population structure and low genetic variation in relatedness among individuals.** Heatmap of the results of hierarchical clustering of the genomic data, with a dendrogram laid down on the top and left showing the kinship matrix for the 399 cultivars of *Vitis vinifera* (399 cultivars listed to the right and bottom of figure). Red coloration indicates most closely related (shown by the red diagonal line running from the bottom left to top right of figure where the comparison between the same cultivar matches up). Yellow coloration indicates not closely related cultivars.

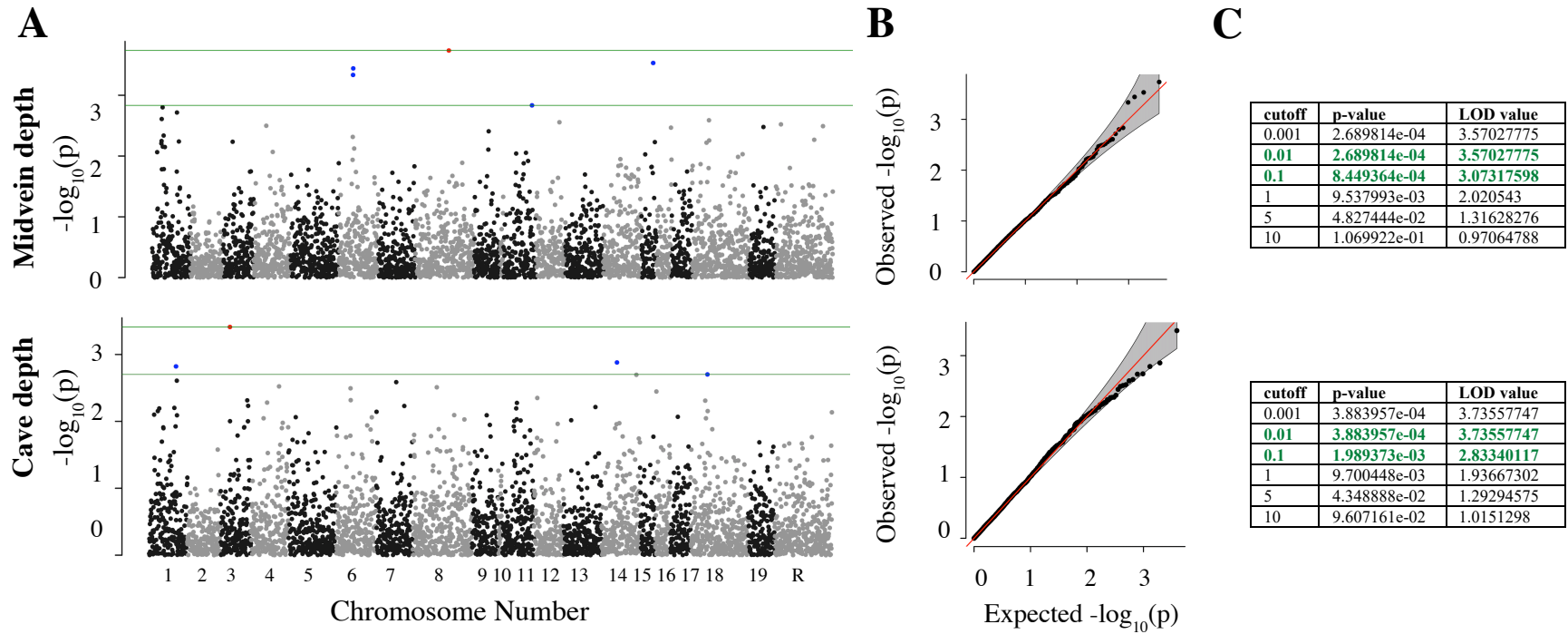


### ***Significant Single-Nucleotide Polymorphisms and Candidate Gene Identification***

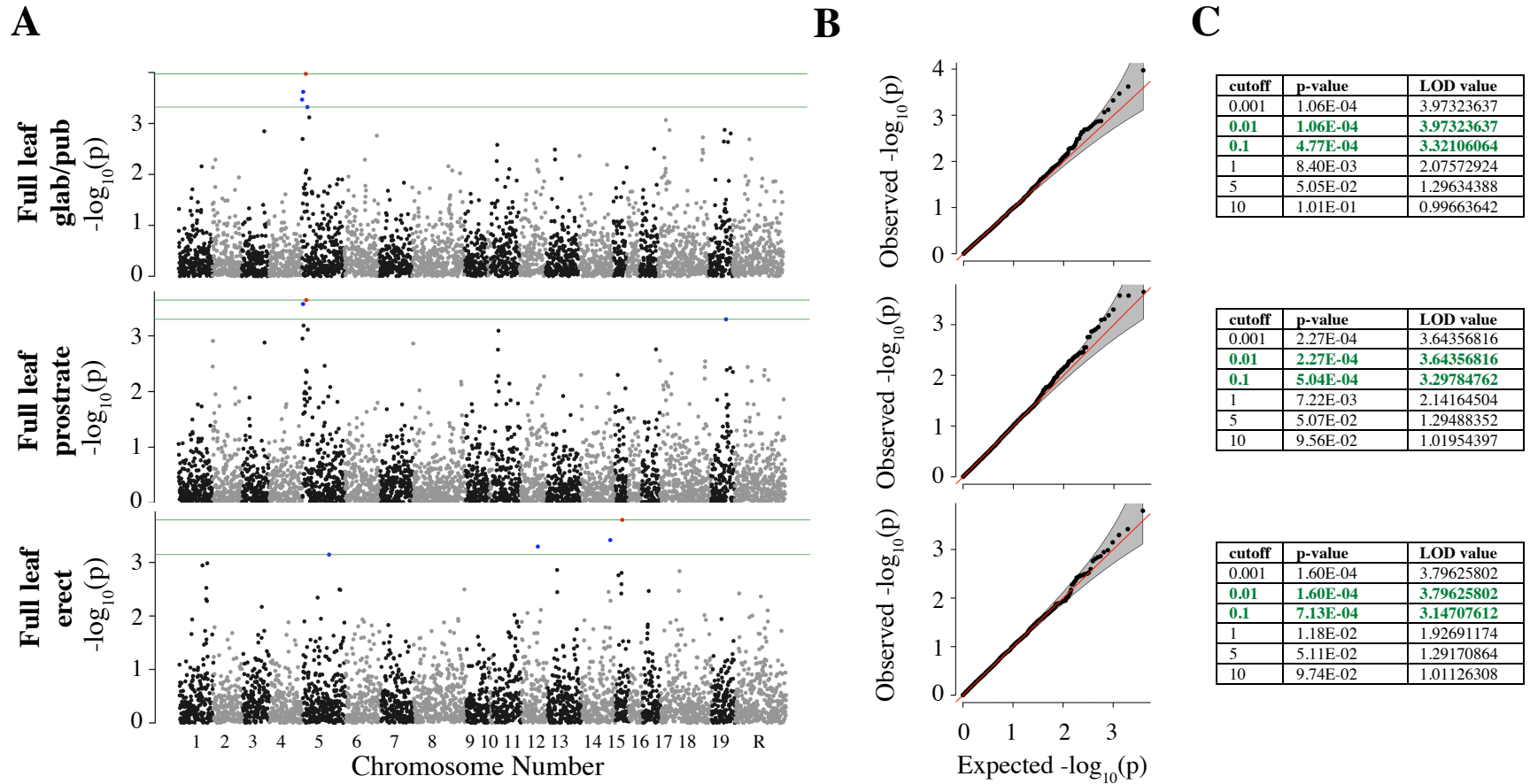
We found multiple significant SNPs associated with each of the eight traits that we measured (Tables 3 & 4; Figures 7.1 & 7.2). All eight phenotypic traits had only a single SNP (red dot) pass the most stringent p-value cutoff and between three and four SNPs (blue dots) that passed the second most stringent p-value cutoff. Several SNPs and chromosomes came up multiple times in our findings. One SNP (chromosome 5, position 1160194) passed the most stringent p-value cutoff for four phenotypic traits (domatia size index, prostrate hair domatia size index, presence/absence of abaxial leaf trichomes, and presence/absence of prostrate hair on abaxial leaf surface) (Tables 3 & 4). Another SNP (chromosome 5, position 725119) passed the less stringent p-value cutoff for both prostrate hair domatia size index and presence/absence of abaxial leaf trichomes (Tables 3 & 4). The presence/absence of total abaxial leaf hairiness had all four SNPs that passed the most stringent and less stringent p-value cutoffs on chromosome 5 (positions 1160194, 725119, 228237, and 1380424) (Table 4). The presence/absence of erect hair on abaxial leaf surface was associated with SNPs on chromosomes 15, 14, 12, and 5 (Table 4). Erect hair domatia size index was not associated with chromosome 5, and instead associated with SNPs on chromosomes 4, 8, and 12 (Table 3). Midvein depth and domatia depth traits were not associated with chromosome 5, and instead associated with SNPs on chromosomes 8, 15, 6, 11 and 3, 14, 1, 18, respectively (Table 3).



**Figure 7.1. GWAS results for the traits investigated in this study.** **A.** Manhattan Plots for each Phenotype. Manhattan plot.  $-\log$  P-values are plotted against physical map position of SNPs. Chromosomes have different colors, and simulated bootstrapping thresholds are marked with green horizontal lines  $p = (0.01, 0.001)$ . **B.** Quantile–quantile (QQ) Plots for each Phenotype. QQ plot determines how GWAS results compare to the expected results under the null hypothesis of no association. **C.** Bootstrapping results. Bootstrapping thresholds are indicated by green text and correspond to the green horizontal lines in the corresponding Manhattan Plot.



**Figure 7.2. GWAS results for the traits investigated in this study.** **A.** Manhattan Plots for each Phenotype. Manhattan plot.  $-\log$  P-values are plotted against physical map position of SNPs. Chromosomes have different colors, and simulated bootstrapping thresholds are marked with green horizontal lines. **B.** Quantile–quantile (QQ) Plots for each Phenotype. QQ plot determines how GWAS results compare to the expected results under the null hypothesis of no association. **C.** Bootstrapping results. Bootstrapping thresholds are indicated by green text and correspond to the green horizontal lines in the corresponding Manhattan Plot.



**Figure 7.3. GWAS results for the traits investigated in this study.** **A.** Manhattan Plots for each Phenotype. Manhattan plot.  $-\log_{10}$  P-values are plotted against physical map position of SNPs. Chromosomes have different colors, and simulated bootstrapping thresholds are marked with green horizontal lines  $p = (0.01, 0.001)$ . **B.** Quantile–quantile (QQ) Plots for each Phenotype. QQ plot determines how GWAS results compare to the expected results under the null hypothesis of no association. **C.** Bootstrapping results. Bootstrapping thresholds are indicated by green text and correspond to the green horizontal lines in the corresponding Manhattan Plot.

**Table 3. Significant SNPs for each phenotype measured with continuous data.** LOD is calculated by taking the negative log base ten of the p-value. The SNPs in red were above the 0.01 significance threshold. All other SNPs passed the 0.1 significant threshold. \*Chromosome R is the assigned placeholder for all unassigned contigs.

| SNP         | Chromosome | Position | P-value    | LOD        | Phenotype                    |
|-------------|------------|----------|------------|------------|------------------------------|
| 8:14233104  | 8          | 14233104 | 0.00018383 | 3.73557747 | Midvein depth                |
| 15:7032829  | 15         | 7032829  | 0.00029477 | 3.53051728 | Midvein depth                |
| 6:6038243   | 6          | 6038243  | 0.00036204 | 3.44123801 | Midvein depth                |
| 6:6036739   | 6          | 6036739  | 0.00046375 | 3.33371462 | Midvein depth                |
| 11:12755911 | 11         | 12755911 | 0.00146757 | 2.83340132 | Midvein depth                |
| 3:2552380   | 3          | 2552380  | 0.0003884  | 3.41072564 | Cave depth                   |
| 14:9932723  | 14         | 9932723  | 0.00131717 | 2.88035704 | Cave depth                   |
| 1:8841287   | 1          | 8841287  | 0.00150833 | 2.82150467 | Cave depth                   |
| 18:4072483  | 18         | 4072483  | 0.00198937 | 2.70128384 | Cave depth                   |
| 5:1160194   | 5          | 1160194  | 0.00026898 | 3.57027775 | Domatia size index           |
| 1:1644108   | 1          | 1644108  | 0.00063555 | 3.19685341 | Domatia size index           |
| R:1074858   | R          | 1074858  | 0.00066383 | 3.17794192 | Domatia size index           |
| 14:2051009  | 14         | 2051009  | 0.00084494 | 3.07317599 | Domatia size index           |
| 4:4681194   | 4          | 4681194  | 0.00020517 | 3.68788891 | Erect domatia size index     |
| 4:1558548   | 4          | 1558548  | 0.00031108 | 3.50712257 | Erect domatia size index     |
| 8:14231408  | 8          | 14231408 | 0.00040946 | 3.38778723 | Erect domatia size index     |
| 12:6506582  | 12         | 6506582  | 0.00093272 | 3.03024996 | Erect domatia size index     |
| 8:15696449  | 8          | 15696449 | 0.00113572 | 2.9447284  | Erect domatia size index     |
| 5:1160194   | 5          | 1160194  | 0.00012812 | 3.89237388 | Prostrate domatia size index |
| 15:3024281  | 15         | 3024281  | 0.00014195 | 3.84785066 | Prostrate domatia size index |
| 19:11137526 | 19         | 11137526 | 0.00035383 | 3.451202   | Prostrate domatia size index |
| 15:6785504  | 15         | 6785504  | 0.00054536 | 3.26331507 | Prostrate domatia size index |
| 5:725119    | 5          | 725119   | 0.00056216 | 3.25014203 | Prostrate domatia size index |

**Table 4. Significant SNPs for each phenotype measured with discrete data.** LOD is calculated by taking the negative log base ten of the p-value. The SNPs in red were above the 0.01 significance threshold. All other SNPs passed the 0.1 significant threshold.

| SNP         | Chromosome | Position | P-value    | LOD        | Phenotype           |
|-------------|------------|----------|------------|------------|---------------------|
| 15:5102580  | 15         | 5102580  | 0.00015986 | 3.79625806 | Full leaf erect     |
| 14:16908983 | 14         | 16908983 | 0.0003811  | 3.41895704 | Full leaf erect     |
| 12:8636792  | 12         | 8636792  | 0.00050402 | 3.29754808 | Full leaf erect     |
| 5:9234333   | 5          | 9234333  | 0.00071273 | 3.14707612 | Full leaf erect     |
| 5:1160194   | 5          | 1160194  | 0.00010636 | 3.97323643 | Full leaf glab/pub  |
| 5:725119    | 5          | 725119   | 0.00023979 | 3.62016916 | Full leaf glab/pub  |
| 5:228237    | 5          | 228237   | 0.00034044 | 3.46795692 | Full leaf glab/pub  |
| 5:1380424   | 5          | 1380424  | 0.00047746 | 3.32106068 | Full leaf glab/pub  |
| 5:1160194   | 5          | 1160194  | 0.00022721 | 3.64356809 | Full leaf prostrate |
| 5:355078    | 5          | 355078   | 0.00026778 | 3.57221772 | Full leaf prostrate |
| 5:355069    | 5          | 355069   | 0.00026778 | 3.57221772 | Full leaf prostrate |
| 19:7824922  | 19         | 7824922  | 0.00050368 | 3.29784766 | Full leaf prostrate |

Gene annotations revealed a suite of potentially relevant genes in proximity to the associated SNPs, including several genes related to trichome development and patterning (Supplementary Tables 3, 5, 6, and 8). We found a SNP (chromosome 5, position 1160194) near a grape gene that has sequence similarity to an Arabidopsis gene that encodes for Trichome Birefringence-Like 4, a gene involved in trichome development. This SNP is associated with several prostrate trichome related traits: two domatia traits (domatia size index and prostrate domatia size index) and two laminar trichome traits (presence/absence of total abaxial leaf hairiness and presence/absence of prostrate hair in full abaxial leaf hairiness). We found SNPs (chromosome 5, position 1160194 & chromosome 5, position 1380424) near grape genes that have sequence similarities to an Arabidopsis gene (outlined in Supplemental Table 9) annotated as MLP-like protein 423, a gene involved in various functions. These SNPs are significantly associated with domatia size, prostrate domatia size, and full leaf glab/pub. Midvein depth and cave depth SNP associations were in close proximity to a handful of annotated genes, none of which had a clear functional tie to these phenotypes (Supplementary Tables 1-2). A full list of gene annotations within a 150kb window up and down-stream of each SNP is provided in Supplementary Tables 1-8.

## DISCUSSION

Plant traits that attract beneficial mites to leaves are widespread across plant species and provide indirect defense via the reduction of pathogens and pests (Weber et al., 2012 & Bronstein, Alarcón, and Geber, 2006). Despite the economic and ecological importance, few studies have investigated the genetic underpinnings of these traits. We examined the genetic associations of mite recruitment traits on *V. vinifera*, a system with strong ecological data linking leaf phenotypes to mite defense. In a GWAS of 399 cultivars from a common garden, we found significant SNPs associated with each of eight mite recruitment traits investigated, including leaf domatia size, vein and cave depth, and leaf trichome traits. Corresponding gene annotations within 150kb up and downstream of these genetic coordinates revealed notable gene associations. In particular, two genes - Trichome Birefringence-Like 4 and Major Latex Protein-like protein 423 - were each associated with four trichome related traits (Supplementary Table 9), suggesting that these genes may have a large impact on mediating mite-plant interactions in this group. Despite the wide-spread distribution of mite-defense in plants, this is the first GWAS conducted on leaf domatia, and one of only a few studies investigating the genetic underpinnings of mite defense traits more generally. Our findings provide rare insight into the genetic drivers of plant-mite mutualism and suggest promising candidates for future studies focused on transcriptomics and ultimately, breeding and genetic editing to increase natural predator-based defenses in *Vitis*.

Several traits related to prostrate leaf trichomes in our dataset associated with a SNP close to the gene TBL4 from *A. thaliana*. This gene codes for the protein Trichome Birefringence-Like 4. The function of this gene is to act as a bridging protein that binds pectin and other cell wall polysaccharides. It is likely involved in maintaining esterification of pectins



and may be involved in the specific O-acetylation of cell wall polymers (Gaudet et al., 2011). The gene's impact on trichome development was illustrated in a knockdown study, which found that the key characteristic of an *A. thaliana* trichome birefringence mutant was severely reduced crystalline cellulose in trichomes (Bischoff et al., 2010). Cellulose crystallinity is a critical component of microstructural parameters that affect the mechanical properties of natural plant fibers and provides significantly more stiffness than all other cellulose constituents contributing to greater structural reinforcement (Fan and Fu, 2016). This is the first study to link TBL4 to a potential role in mite defense. Given the repeated strong association of TBL4 to prostrate-trichome related traits in *V. vinifera*, and the clear ecological evidence linking prostrate hair phenotypes to mite recruitment in *Vitis*, this gene merits further investigation for a potential role in mediating indirect defense in grape.

Although each of our trichome-related phenotypes were significantly associated with SNPs in the *V. vinifera* panel, there were no additional gene annotations related to trichome development in close proximity to significant SNPs beyond TBL4. This could be due to a lack of annotations or the result of genes that have multiple functions annotated with only part of their functionality. Domatia development in *Vitis* could also involve genes that are not related to trichome development explored in *A. thaliana*. Aside from Trichome Birefringence-like 4, we found many gene annotations that were shared across trichome phenotypes (Supplementary Table 3, 5, 6, and 8). The most notable, was a single gene annotation, Major Latex Protein (MLP)-like protein 423, that was associated with four different phenotypes (domatia size index, prostrate hair domatia size index, presence/absence of prostrate hair in full abaxial leaf hairiness, and presence/absence of total abaxial leaf hairiness). This major latex protein is documented to be involved in response to biotic stimulus and defense response (UniProt Consortium, 2019). A

defense response is triggered in response to the presence of a foreign body or the occurrence of an injury (like a biotic stimulus), which results in restriction of damage to the organism attacked or prevention/recovery from the infection caused by the attack (UniProt Consortium, 2019).

Research on the MLP-like protein 423 in *Vitis* found the protein was related to plant hormone signal transduction pathways in bud dormancy (Khalil-Ur-Rehman et al., 2017), candidate regulatory and functional genes involved in cold stress (Xu et al., 2014), and lipid associated signaling involved in defense against *Plasmopara viticola* (Figueiredo et al., 2016). While we cannot establish a direct link between these genes and mite defense in *Vitis*, our results highlight the genes coding for MLP-like protein 423 as strong candidates for future studies interested in genetic and developmental drivers of mite defense phenotypes in this group.

Both of our non-trichome related phenotypes, cave depth and midvein depth, were also significantly associated with SNPs in the *V. vinifera* panel. Notable gene annotations for cave depth associated with a SNP on chromosome 18 (position 4072483) included AGD2-like defense response protein 1 and Pollen Ole e 1 allergen and extensin family protein. AGD2-like defense response protein 1 encodes aminotransferases that act on an overlapping set of amino acids that promotes development and suppresses defenses (Song, Lu, and Greenberg, 2004). This locus is also associated with the tocopherol biosynthetic pathway in *Arabidopsis*, that influences salicylic acid biosynthesis and guarantees effective basal resistance against *Pseudomonas syringae* bacteria (Stahl et al., 2019). The Pollen Ole e 1 allergen and extensin family protein is expressed in leaves and is associated with host pathogen response and cell cycle changes in *Arabidopsis*, during geminivirus infection (Ascencio-Ibáñez et al., 2008). Notable gene annotations for midvein depth, associated with a SNP on chromosome 6 (position 6038243), included Translocon at the outer envelope membrane of chloroplasts 75-III. This is a component of the

translocon outer membrane (TOC) complex that forms the outer envelope translocation channel (beta-barrel) (Zhang et al., 2018). It plays a role in preprotein conductance, is imported into chloroplasts, and is expressed in young dividing photosynthetic tissues (Zhang et al., 2018). While we cannot establish a direct link between these genes and *Vitis* leaf development characteristics, but our results highlight the genes coding for defense response proteins and photosynthetic tissues as strong candidates for future studies interested in genetic and developmental drivers of leaf development and pathogen resistance phenotypes in *Vitis*.

While our study is the first investigation of the genetic drivers of mite-recruitment phenotypes in *V. vinifera*, and the first GWAS conducted of leaf domatia more generally, other work has examined mite related phenotypes using alternative genetic approaches in *Vitis* (Barba et al 2019). Barba et al. (2019) conducted a QTL association study examining mite related traits across crosses of two *Vitis* hybrids. Their work provided evidence for a major locus influencing trichome densities, domatia size, and predatory mite abundance on *Vitis* hybrids. Interestingly, none of our significant SNPs were in close proximity (150kb) of candidate genes identified by Barba et al., including either of our significant findings for Trichome Birefringence-Like 4 or MLP-like protein 423. This suggests domatia and trichome development could occur via different genetic pathways in different *Vitis* species. Alternatively, variation in findings could also be attributed to different methodologies (GWAS vs QTL) despite using the same reference genome (i.e. the parental lines in the hybrids may not have had the same variation as our SNPs, an especially likely scenario given the low numbers of SNPs identified). Further work is needed to determine whether domatia and trichome development occurs via divergent genetic mechanisms across *Vitis*.

Although our study presents exciting candidates for future work focused on the genetics of mite-plant interaction traits, there are several important caveats to consider. The *V. vinifera* used in this study was clonally propagated according to standard agricultural practices. As a result, our panel had high linkage disequilibrium, lacked outgroups, and had high genetic relatedness that can lead to a high rate of false positive associations (Sul, Martin, and Eskin, 2018). While our panel covered a diversity of cultivar genotypes and captured variability in our key phenotypes, additional sampling (both within and across cultivars) would have strengthened our study. Finally, although we focused on traits with strong previous research linking phenotypes to mite defense, we did not directly assess that link in this study. Future work aimed at experimentally linking genetic variation in candidate loci to mite defense is a natural follow up to this work. Future work should also aim to sequence the genes found in this GWAS and the surrounding areas in the cultivars with the most extreme phenotypes to validate them as candidate genes.

Ultimately, this study represents a key step in determining the genetic drivers of an important, but relatively understudied mutualistic interaction between beneficial mites and plants. We were able to determine candidate regions for driving mite recruitment traits on *Vitis vinifera* and found significant SNPs associated with each of the eight mite recruitment traits we investigated, including leaf domatia size, vein and cave depth, and full leaf trichome traits. Corresponding gene annotations of these genetic coordinates revealed notable gene associations including Trichome Birefringence-Like 4 and MLP-like protein 423, both associated with four traits, suggesting that these genes may have a large impact mediating mite-plant interactions in this species. The domesticated grape is one of the most economically important perennial fruit crops in the world, generating \$136.6B in trade in 2018 (Research and Markets, 2020) and

utilizing more than 7.4 million hectares of land in both temperate and tropical climatic regions around the world for the production of table grapes, raisins, juice, wine, and spirits (Adam-Blondon et al., 2016). This research increases our knowledge of grapevine biology, paving the way to support the development of new cultivars adapted to more sustainable viticulture. Our findings suggest promising candidates for future studies focused on transcriptomics, breeding and genetic editing to increase naturally occurring predator-based defenses already in place in *Vitis*. More generally, this study is among the first to provide a rare look into the genetic drivers of traits underlying indirect defense of plants via beneficial mites in any system. Future molecular and experimental work building on our results can help inform our understanding of the genes associated with plant-mite defense interactions in nature.

## REFERENCES

## REFERENCES

- Adam-Blondon, Anne-Françoise, Jose-Miguel Martinez-Zapater, and Chittaranjan Kole, eds. *Genetics, Genomics, and Breeding of Grapes*. CRC Press, 2016.
- Agrawal, Anurag A., and Mark Fishbein. "Plant defense syndromes." *Ecology* 87.sp7 (2006): S132-S149.
- Agrawal, Anurag A., Richard Karban, and Ramana G. Colfer. "How leaf domatia and induced plant resistance affect herbivores, natural enemies and plant performance." *Oikos* 89.1 (2000): 70-80.
- Ascencio-Ibáñez, José Trinidad, et al. "Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection." *Plant physiology* 148.1 (2008): 436-454.
- Balkunde, Rachappa, Martina Pesch, and Martin Hülskamp. "Trichome patterning in Arabidopsis thaliana: from genetic to molecular models." *Current topics in developmental biology*. Vol. 91. Academic Press, 2010. 299-321.
- Barba, Paola, et al. "A QTL associated with leaf trichome traits has a major influence on the abundance of the predatory mite Typhlodromus pyri in a hybrid grapevine population." *Horticulture research* 6.1 (2019): 1-12.
- Becker, R. A., J. M. Chambers, and A. R. Wilks. "The New S Language. Wadsworth & Brooks/Cole." *Computer Science Series, Pacific Grove, CA* (1988).
- Bischoff, Volker, et al. "TRICHOME BIREFRINGENCE and its homolog AT5G01360 encode plant-specific DUF231 proteins required for cellulose biosynthesis in Arabidopsis." *Plant physiology* 153.2 (2010): 590-602.
- Bilyeu, Kristin, Milind B. Ratnaparkhe, and Chittaranjan Kole, eds. *Genetics, genomics, and breeding of soybean*. CRC Press, 2016.
- Bonferroni, Carlo E., C. Bonferroni, and C. E. Bonferroni. "Teoria statistica delle classi e calcolo delle probabilita'." (1936).
- British Columbia: Ministry of Agriculture. Grape Powdery Mildew. Province of British Columbia. (2015). <https://www2.gov.bc.ca/gov/>
- Bronstein, Judith L., Ruben Alarcón, and Monica Geber. "The evolution of plant–insect mutualisms." *New Phytologist* 172.3 (2006): 412-428.

- Brouwer, Yvonne Marguerite, and Harold Trevor Clifford. "An annotated list of domatia-bearing species." *Notes from the Jodrell Laboratory* 12 (1990): 1-33.
- De Moraes, Consuelo M., et al. "Herbivore-infested plants selectively attract parasitoids." *Nature* 393.6685 (1998): 570-573.
- Duso, Carlo, et al. "Is the predatory mite *Kampimodromus aberrans* a candidate for the control of phytophagous mites in European apple orchards?" *BioControl* 54.3 (2009): 369-382.
- English-Loeb, Greg, Andrew P. Norton, and M. Andrew Walker. "Behavioral and population consequences of acarodomatia in grapes on phytoseiid mites (Mesostigmata) and implications for plant breeding." *Entomologia Experimentalis et Applicata* 104.2-3 (2002): 307-319.
- English-Loeb, Greg, et al. "Tri-trophic interactions among grapevines, a fungal pathogen, and a mycophagous mite." *Ecological Applications* 15.5 (2005): 1679-1688.
- English-Loeb, Greg, et al. "Biological control of grape powdery mildew using mycophagous mites." *Plant disease* 91.4 (2007): 421-429.
- Fatouros, Nina E., et al. "Male-derived butterfly anti-aphrodisiac mediates induced indirect plant defense." *Proceedings of the National Academy of Sciences* 105.29 (2008): 10033-10038.
- Fan, Mizi, and Feng Fu, eds. *Advanced high strength natural fibre composites in construction*. Woodhead Publishing, 2016.
- Figueiredo, Andreia, et al. "Specific adjustments in grapevine leaf proteome discriminating resistant and susceptible grapevine genotypes to *Plasmopara viticola*." *Journal of proteomics* 152 (2017): 48-57.
- Korte, Arthur, and Ashley Farlow. "The advantages and limitations of trait analysis with GWAS: a review." *Plant methods* 9.1 (2013): 29.
- Gago, P., et al. "Microanatomy of leaf trichomes: opportunities for improved ampelographic discrimination of grapevine (*Vitis vinifera* L.) cultivars." *Australian journal of grape and wine research* 22.3 (2016): 494-503.
- Galet, P. "A practical Ampelography (Grapevine identification) Luicie T." *Morton, Leon D. Adams* (1979).
- Gaudet, Pascale, et al. "Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium." *Briefings in bioinformatics* 12.5 (2011): 449-462.
- Grostal, Raul, and Dennis J. O'Dowd. "Plants, mites and mutualism: leaf domatia and the abundance and reproduction of mites on *Viburnum tinus* (Caprifoliaceae)." *Oecologia* 97.3 (1994): 308-315.



- Hanley, Mick E., et al. "Plant structural traits and their role in anti-herbivore defence." *Perspectives in Plant Ecology, Evolution and Systematics* 8.4 (2007): 157-178.
- Holeski, Liza M., Ronnette Chase-Alone, and John K. Kelly. "The genetics of phenotypic plasticity in plant defense: trichome production in *Mimulus guttatus*." *The American Naturalist* 175.4 (2010): 391-400.
- Holm, Sture. "A simple sequentially rejective multiple test procedure." *Scandinavian journal of statistics* (1979): 65-70.
- IPGRI, U., 1997. OIV. 1997. *Descriptors for Grapevine (Vitis spp.)*. International Union for the Protection of New Varieties of Plants, Geneva, Switzerland/Office International de la Vigne et du Vin, Paris, France/International Plant Genetic Resources Institute, Rome, Italy (Vol. 142, No. 34, p. 4).
- Jaillon, Olivier, et al. "The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla." *nature* 449.7161 (2007): 463.
- Karban, Richard, et al. "Abundance of phytoseiid mites on *Vitis* species: effects of leaf hairs, domatia, prey abundance and plant phylogeny." *Experimental & applied acarology* 19.4 (1995): 189-197.
- Khalil-Ur-Rehman, Muhammad, et al. "Comparative RNA-seq based transcriptomic analysis of bud dormancy in grape." *BMC plant biology* 17.1 (2017): 18.
- Korte, Arthur, and Ashley Farlow. "The advantages and limitations of trait analysis with GWAS: a review." *Plant methods* 9.1 (2013): 29.
- Leiserson, Mark DM, et al. "Network analysis of GWAS data." *Current opinion in genetics & development* 23.6 (2013): 602-610.
- Lipka, Alexander E., et al. "GAPIT: genome association and prediction integrated tool." *Bioinformatics* 28.18 (2012): 2397-2399.
- Loughner, R., et al. "Influence of leaf trichomes on predatory mite (*Typhlodromus pyri*) abundance in grape varieties." *Experimental and Applied Acarology* 45.3-4 (2008): 111-122.
- Mamidi, Sujana, et al. "Genome-wide association studies identifies seven major regions responsible for iron deficiency chlorosis in soybean (*Glycine max*)." *PloS one* 9.9 (2014).
- Marks, M. David, and Kenneth A. Feldmann. "Trichome development in *Arabidopsis thaliana*. I. T-DNA tagging of the GLABROUS1 gene." *The plant cell* 1.11 (1989): 1043-1050.
- McGovern, Patrick E. *Ancient wine: the search for the origins of viniculture*. Princeton University Press, 2019.

- Melidossian, Heather S., et al. "Suppression of grapevine powdery mildew by a mycophagous mite." *Plant disease* 89.12 (2005): 1331-1338.
- Mooney, Kailen A., and Anurag A. Agrawal. "Plant genotype shapes ant-aphid interactions: implications for community structure and indirect plant defense." *The American Naturalist* 171.6 (2008): E195-E205.
- Myles, Sean, et al. "Genetic structure and domestication history of the grape." *Proceedings of the National Academy of Sciences* 108.9 (2011): 3530-3535.
- Myles, Sean, et al. "Rapid genomic characterization of the genus vitis." *PloS one* 5.1 (2010).
- Norton, Andrew P., et al. "Mycophagous mites and foliar pathogens: leaf domatia mediate tritrophic interactions in grapes." *Ecology* 81.2 (2000): 490-499.
- O'Dowd, Dennis J., and Mary F. Willson. "Leaf domatia and the distribution and abundance of foliar mites in broadleaf deciduous forest in Wisconsin." *American Midland Naturalist* (1997): 337-348.
- Pemberton, Robert W., and Charles E. Turner. "Occurrence of predatory and fungivorous mites in leaf domatia." *American Journal of Botany* 76.1 (1989): 105-112.
- Quintero, Carolina, Kasey E. Barton, and Karina Boege. "The ontogeny of plant indirect defenses." *Perspectives in plant ecology, evolution and systematics* 15.5 (2013): 245-254.
- Team, R. Core. "R: A language and environment for statistical computing." (2013): 201.
- Research and Markets. (2020). World Grapes Market Analysis, Forecast, Size, Trends and Insights. (Report No. 4701076). Retrieved from <https://www.researchandmarkets.com/reports/4701076/>
- Revelle, William. "psych: Procedures for psychological, psychometric, and personality research." *Northwestern University, Evanston, Illinois* 165 (2014): 1-10.
- Russo, Pedro ST, et al. "CEMiTool: a Bioconductor package for performing comprehensive modular co-expression analyses." *Bmc Bioinformatics* 19.1 (2018): 56.
- Song, Jong Tae, Hua Lu, and Jean T. Greenberg. "Divergent roles in Arabidopsis thaliana development and defense of two homologous genes, aberrant growth and death2 and AGD2-LIKE DEFENSE RESPONSE PROTEIN1, encoding novel aminotransferases." *The Plant Cell* 16.2 (2004): 353-366.
- Stahl, Elia, et al. "A Role for Tocopherol Biosynthesis in Arabidopsis Basal Immunity to Bacterial Infection." *Plant physiology* 181.3 (2019): 1008-1028.

- Sul, Jae Hoon, Lana S. Martin, and Eleazar Eskin. "Population structure in genetic studies: Confounding factors and mixed models." *PLoS genetics* 14.12 (2018).
- UniProt Consortium. "UniProt: a worldwide hub of protein knowledge." *Nucleic acids research* 47.D1 (2019): D506-D515.
- VanRaden, Paul M. "Efficient methods to compute genomic predictions." *Journal of dairy science* 91.11 (2008): 4414-4423.
- Weber, Marjorie G., et al. "Phylogenetic and experimental tests of interactions among mutualistic plant defense traits in *Viburnum* (Adoxaceae)." *The American Naturalist* 180.4 (2012): 450-463.
- Wei, Taiyun, and Viliam Simko. "R package" corrplot": Visualization of a Correlation Matrix (Version 0.84)." *Retrieved from, <https://github.com/taiyun/corrplot>* (2017).
- Xu, Weirong, et al. "Transcriptome profiling of *Vitis amurens*, an extremely cold-tolerant Chinese wild *Vitis* species, reveals candidate genes and events that potentially connected to cold stress." *Plant molecular biology* 86.4-5 (2014): 527-541.
- Yu, Jianming, et al. "A unified mixed-model method for association mapping that accounts for multiple levels of relatedness." *Nature genetics* 38.2 (2006): 203-208.
- Zhang, Xiaolan, et al. "Auxin response gene *SlARF3* plays multiple roles in tomato development and is involved in the formation of epidermal cells and trichomes." *Plant and Cell Physiology* 56.11 (2015): 2110-2124.
- Zhang, Shoudong, et al. "The caseinolytic protease complex component CLPC1 in *Arabidopsis* maintains proteome and RNA homeostasis in chloroplasts." *BMC plant biology* 18.1 (2018): 192.
- Zhang, Zhiwu, et al. "Mixed linear model approach adapted for genome-wide association studies." *Nature genetics* 42.4 (2010): 355.