Title: Host transfer triggers widespread plasticity in gene expression, but not DNA methylation, in a specialist herbivore

Running Title: Transcriptional plasticity enables host use

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Abstract

Insect herbivores employ many adaptive mechanisms to successfully colonize host plants. Transcriptional plasticity is one mechanism that enables broad host use in polyphagous insect herbivores; however, whether specialist herbivores use transcriptional plasticity or undergo epigenome modifications to cope with host shifts remains largely unexplored. We measured the transcriptome and epigenome of the specialist aphid herbivore, *Daktulosphaira vitifoliae*, after transfer to new hosts using RNA-seq and whole-genome bisulfite sequencing. We found the transcriptome of *D. vitifoliae* is highly plastic and responsive to host genotype – 3,706 genes (28% of expressed genes) are significantly differentially expressed in at least one contrast comparing host genotypes. Most host-responsive genes (2,667 genes) were upregulated when feeding on the non-native host *Vitis vinifera* and enriched for genes encoding predicted secretory effectors, detoxification enzymes, and cuticle- and chitin-associated proteins. We found gene co-expression modules that correlated with variation in fitness suggesting plasticity in transcriptional networks contributes to *D.*

vitifoliae adaptation to host plants. We also identified a previously undescribed family of proline-rich secretory proteins conserved across the Aphidomorpha infraorder of insects. Unlike the transcriptome, DNA methylation did not dramatically respond to host transfer and positively correlated with stably expressed genes; thus, gene body DNA methylation may help stabilize gene expression when insects move to new host environments. Together, these results provide new insight into the molecular mechanisms of host use in insect herbivores.

Key Words: adaptation, aphids, effectors, grapevines, phenotypic plasticity, methylome

Introduction

Phenotypic plasticity, or the expression of different phenotypes by the same genotype, can allow organisms to flexibly respond to environmental changes, and has thus been widely proposed to contribute to the evolution of species (Ancel, 2000; Pigliucci & Murren, 2003; Ghalambor et al., 2007; Murren et al., 2015). However, the extent to which plasticity alters adaptative evolution remains an open question (Vinton et al., 2022). Insect herbivores are particularly good systems for understanding the mechanisms of plasticity and adaptation, given their ecological diversity (Futuyma & Agrawal, 2009; Forister et al., 2015), ability to rapidly adapt to new environments (Garnas, 2018), and dramatic examples of environment-induced phenotypic variation (Whitman & Agrawal, 2009). Although plant–herbivore interactions, and evolutionary ecology more broadly, are well established fields within evolutionary biology, the molecular mechanisms that underlie species interactions are only beginning to be understood.

Insect herbivores are among the most species-rich lineages of metazoans, and this diversity is largely driven by adaptations to specific host plants (Simon et al., 2015). These adaptations function as a mode of ecological specialization that induces trade-offs in fitness when an herbivorous insect transitions from ancestral to alternative hosts (García-Robledo & Horvitz, 2012). Support for this hypothesis comes from the fact that most insect herbivores are monophagous or oligophagous (i.e. specialists), feeding on

one or very few species of closely related hosts (Bernays & Graham, 1988; Futuyma & Agrawal, 2009; Forister et al., 2015). For polyphagous, generalist herbivores, compatibility across a breadth of divergent hosts is hypothesized to be maintained by a high degree of genomic flexibility (Sexton et al., 2017; Birnbaum & Abbot, 2020). Indeed, the genomes of generalist insect herbivores contain extraordinary expansions of chemoreception and detoxification genes (W. Xu et al., 2016; Grbić et al., 2011; Pearce et al., 2017), which enable host finding and feeding on plants. In contrast, specialist herbivores appear to lack such expansions of chemoreception and detoxification genes (Peláez et al., 2023). Additionally, there is increasing evidence that both generalists and specialist herbivores rely on expanded families of secretory effector proteins to directly manipulate host plant biology during feeding (Zhao et al., 2015; Mathers et al., 2017; Rispe et al., 2020; Korgaonkar et al., 2021).

Transcriptional plasticity of expanded gene families has been linked to fitness variation in generalist insect herbivores during interactions with divergent host plants (Mathers et al., 2017; Snoeck et al., 2018), suggesting transcriptional plasticity plays an adaptive role in these insects. Most studies that investigated transcriptional responses of insects to different host plants used generalists, as these insects typically represent major agricultural pests (Grbić et al., 2011; Mathers et al., 2017; Pearce et al., 2017). Transcriptional plasticity is hypothesized to be reduced in specialist herbivores relative to generalists (Birnbaum & Abbot, 2020), given host-specific evolution, i.e. reduced diet breadth. Indeed, the few studies that measured transcriptomic responses to host transfer in both specialist and generalist insects found overall reduced transcriptional plasticity in specialists (Govind et al., 2010; Roy et al., 2016; Schweizer et al., 2017). From an evolutionary perspective, specialization and the reduced capacity for plasticity are expected to come at a cost, limiting adaptability of species amidst rapid environmental change (Futuyma & Moreno, 1988; Price et al., 2003; Forister et al., 2012; Vamosi et al., 2014; Murren et al., 2015; Day et al., 2016).

Dynamic regulation of the epigenome is thought to be a central mechanism underlying rapid, environment-induced transcriptional plasticity (Duncan et al., 2014; Kilvitis et al., 2017; Duncan et al., 2022). DNA methylation of cytosines, the covalent addition of a methyl group to these residues, is a well-known epigenetic mark that

maintains functional gene regulation in many eukaryotic organisms (Zemach et al., 2010; Schmitz et al., 2019). Cytosine methylation, hereafter DNA methylation, has been well-studied in some insect lineages, primarily social Hymenoptera for its role in mediating behavioral and phenotypic plasticity (Yan et al., 2014, 2015). Unlike in plants and mammals, DNA methylation in insects occurs at relatively low levels and is found primarily in gene body regions of actively expressed genes. Interestingly, some insects lack discernable levels of DNA methylation in their genomes, suggesting its role in gene regulation is dispensable (Bewick et al., 2016; Provataris et al., 2018). In insects with abundant DNA methylation the observed variation has been linked to developmental and phenotypic plasticity (Glastad et al., 2014, 2019); however, the direct role of DNA methylation in regulating transcriptional plasticity in insects remains unclear. Early studies in honey bees (*Apis mellifera*) found differential DNA methylation coincides with differential gene expression (Kucharski et al., 2008; Li-Byarlay et al., 2013) whereas recent studies suggest there is no correlation (Harris et al., 2019; Marshall et al., 2019; Cardoso-Júnior et al., 2021). In other insects such as the green peach aphid (*Myzus persicae*), differential DNA methylation correlated strongly with differential expression of sex-linked genes (Mathers et al., 2019), suggesting DNA methylation may regulate transcriptional plasticity and the emergence of different phenotypes. Plasticity in DNA methylation may similarly regulate plasticity in herbivore phenotypes in response to different host plants, although until our study here, no experiments have tested this hypothesis. More broadly, the role of transcriptional plasticity and DNA methylation in herbivore acclimatization and adaptation to hosts remains uncharacterized.

The degree to which phenotypic plasticity, and the underlying epigenetic and transcriptional plasticity, contribute to adaptation and evolution remains contentious (Pigliucci & Murren, 2003; Price et al., 2003; Ghalambor et al., 2007; Vinton et al., 2022). Plasticity may slow the rate of genetic evolution by shielding genotypes from the effects natural selection because one genotype expresses multiple phenotypes across environments (Ancel, 2000; Price et al., 2003; Paenke et al., 2007). Alternatively, plasticity that produces phenotypes which are favored by natural selection may later become genetically assimilated, a case where plasticity results in adaptive evolution (Price et al., 2003; Paenke et al., 2007; Lande, 2009). Whether plasticity is adaptive

125 depends on the strength and direction of selection. Plastic responses may move
126 individuals towards a fitness optimum (adaptative), away from a fitness optimum
127 (maladaptive), or have a neutral effect (Pigliucci & Murren, 2003; Ghalambor et al.,
128 2007). A separate but related question is *to what extent does environment-induced*
129 *plasticity lead to phenotypic adaptation via transgenerational inheritance of epigenetic*
130 *marks*? The answer to this question similarly remains contentious, and likely dependent
131 on the lineage or trait in question. The transfer of DNA methylation marks across
132 generations in mammals seems to be rare, because almost all DNA methylation is
133 erased before being reestablished during embryogenesis (Morgan et al., 2005; H. J.
134 Lee et al., 2014; S.-M. Lee & Surani, 2024). In insects, conversely, DNA methylation
135 erasure during embryogenesis does not occur (Harris et al., 2019; X. Xu et al., 2019),
136 and DNA methylation can be inherited across generations in *Nasonia* wasps (Wang et
137 al., 2016) and honey bees (Yagound et al., 2020). Thus, whether inheritance of
138 epigenetic marks can contribute to phenotypic adaptation in insects remains an open
139 question.
140 In our study, we examined host-induced variation in fitness, gene expression,
141 and DNA methylation in the specialist herbivore *Daktulosphaira vitifoliae* (Fitch). This
142 gall-forming aphid-like insect, commonly known as grape phylloxera, is in the
143 Phylloxeridae family (Aphidomorpha infraorder) of Hemipteran insects and feeds strictly
144 on grapevines (species within the *Vitis* genus of flowering plants) (Granett et al., 2001;
145 Metz et al., 2017). Although native to North America, *D. vitifoliae* has invaded grape
146 growing regions across the globe, becoming one of the most infamous pests of
147 grapevines (Rispe et al., 2020). The accidental introduction into France in the late
148 1800's caused the 'phylloxeric plague', wiping out thousands of vineyards and
149 threatening global grape production (Rispe et al., 2020). While agriculturally relevant, *D.*
150 *vitifoliae* also provides a model study system for ecological genomics and epigenetics.
151 Short generation times of asexually reproducing individuals allow for large clonal
152 populations to be reared and removes any confounding effects of genetic background
153 on transcriptional or epigenetic plasticity, which is a notable advantage in our study. The
154 obligate nature of *D. vitifoliae* should also strengthen the ability to detect how host
155 switching shapes trait plasticity, given the outcome is adapt or die. To explore these

156 traits, we investigate the transcriptional and epigenetic basis of host use in *D. vitifoliae*
157 as it feeds on different host genotypes for a single generation. We test the hypothesis
158 that plastic responses of the transcriptome mediate variation in *D. vitifoliae* fitness on
159 different hosts, such that the direction of plasticity in transcriptional networks is
160 positively correlated (adaptive) or negatively correlated (maladaptive) with fitness
161 variation. We also analyze DNA methylation in the progeny of *D. vitifoliae* adults that fed
162 on different hosts for one generation to characterize the phylloxerid epigenome. We test
163 the hypothesis that environment-induced plasticity leads to predictable changes in the
164 epigenome of *D. vitifoliae* after one generation in different host environments, providing
165 new evidence, or lack thereof, for the role of DNA methylation in phenotypic adaptation
166 in insects.
167
168 **Materials and Methods**
169 Plant and insect rearing
170 A clonal line of *D. vitifoliae* was established from a single female leaf gall
171 collected on *V. rupestris* (cv St. George) near Davis, California U.S.A., in 2021. The
172 clonal population was maintained for successive generations on *V. vinifera* cv Cabernet
173 Sauvignon 33 (Cab33) that were grafted (*Vitis* genotype 1616 and 3309 rootstocks) and
174 self-rooted in UC Riverside greenhouses in Riverside, CA (27 °C +/-3 °C) until
175 experimentation during 2022-2023.
176 We used nine different grapevine genotypes as hosts for *D. vitifoliae* in our
177 experiments, all of which were clonally propagated from cuttings purchased as rooted,
178 overwintered 1-year-old plants from Inland Desert Nursery (Benton City, WA).
179 Genotypes included self-rooted rootstock hybrids of *V. berlandieri* x *V. rupestris* (99R,
180 1103P), *V. berlandieri* x *V. riparia* (SO4, 5BB), and *V. riparia* x *V. rupestris* (101-14, SW,
181 3309). All plants were potted in 11.3 liter round pots with 'UC Mix 3' soil
182 (https://agops.ucr.edu/soil-mixing#uc-soil-mix-iii) and grown in a greenhouse set at
183 24:27 °C +/- 3 °C (night:day) with a 14h photoperiod. After leaf bud swelling (8 days
184 after potting), half of the plants for each genotype were infested. The remaining plants
185 were potted and infested similarly 6 days later. Plantings were replicated through time to
186 optimize sample collection effort given the time required to sort and count insects.

187

188 Host transfer and insect performance assay

189 For experimental infestation of new host plants (host transfer), galled leaves from
190 the colony on Cab33 (hereafter 'G0', for generation 0) were excised and placed on the
191 branch nodes of new host plants. Infestation was monitored every 12 hours to note
192 hatch and dispersal of 1st nymphal instars (hereafter generation 1, 'G1') onto budding
193 leaves. Galled leaves used for infestation were removed from branch nodes after two
194 days to prevent further infestation.

195 To assess insect performance, 'G1' galls were collected 15 days post infestation
196 (DPI). Galls were collected using a 4 mm diameter hole punch and carefully opened
197 under a dissecting microscope to count eggs per gall. Each plant was sampled five
198 different times, at 15, 17, 19, 21, and 23 DPI. Each time, the five largest galls from each
199 plant were sampled, selecting galls from leaves that had not been previously sampled
200 unless the largest remaining galls were on a previously sampled leaf. To determine if *D.*
201 *vitifoliae* fitness varied significantly on different hosts, pairwise Wilcoxon rank-sum tests
202 were performed (R v. 4.3.1).

203

204 RNA isolation and sequencing

205 To quantify *D. vitifoliae* transcriptome plasticity during host transfer events,
206 twenty G1 adults were collected from galls on unique plants and pooled for each
207 sample. Samples were collected 15-23 DPI and placed in tubes on ice during collection,
208 then immediately stored at -80 °C until RNA isolation. RNA was isolated from adults
209 using the RNeasy Mini Kit (Qiagen) following manufacturer's instructions. RNA
210 intactness and purity were verified with gel electrophoresis and spectrophotometry
211 (260:280 > 2; 260:230 >1.7) prior to library preparation using NEBNext® Ultra™ II
212 Directional RNA Library Prep Kit for Illumina® (New England Biolabs). RNA libraries
213 were sequenced using an Illumina NovaSeq X system at the DNA Technologies and
214 Expression Analysis Core of the UC Davis Genome Center, Davis, CA, with an average
215 yield of 28.9 ($\pm$ 3.29 s.d.) million paired-end reads per sample. Sequencing data were
216 uploaded to the NCBI Sequence Read Archive (SRA) repository under Bioproject
217 accession PRJNA1211173.

218

219 RNA-seq data processing

220 Fastqc v. 0.11.9 (https://github.com/s-andrews/FastQC) was used to generate
221 read and base quality statistics for raw and processed reads. Raw RNA-seq reads were
222 quality trimmed and filtered using BBTools bbduk (v.38.95; https://jgi.doe.gov/data-and-
223 tools/software-tools/bbtools/) with default settings plus options for kmer length (k=23)
224 and hamming distance (hist=1). Trimmed and filtered reads were aligned to the
225 reference genome (Rispe et al., 2020) using STAR v. 2.7.10b (Dobin et al., 2013) with
226 default settings plus options for maximum intron length (--alignIntronMax 15000) and
227 removing alignments that contained non-canonical junctions (--outFilterIntronMotifs
228 RemoveNoncanonical). On average, 85.8% ($\pm$ 1.49% s.d.) of reads uniquely mapped to
229 the reference genome, yielding 6.80 Gigabases ($\pm$ 0.74 s.d.) of read coverage (Table
230 S1). Only uniquely mapped reads were used for downstream analysis.

231

232 Differential gene expression analysis

233 Gene count matrices were generated using the --quantMode GeneCounts option
234 in STAR and analyzed using edgeR (v.4.0.16) (Robinson et al., 2010). Low count genes
235 were removed from downstream analysis using filterByExpr with default parameters.
236 Linear model fitting of count data was performed using limma's voom and lmFit
237 functions (Law et al., 2014). Genes were considered differentially expressed based on
238 the empirical Bayes method implemented using limma's 'treat' function with a 1.5-fold-
239 change filter and BH-adjusted p-value < 0.05. Genes that were differentially expressed
240 between 'G1' Cab33 and 'G0' (initial *D. vitifoliae* colony on Cab33) were removed for
241 downstream analysis of host-responsive genes, since these were differentially
242 expressed across generations on the same host genotype. Fisher's exact testing for
243 gene functional group enrichment was performed with the R stats package (v.4.3.1).
244 Functional groups were considered significantly enriched based on a Benjamini
245 Hochberg (BH)-adjusted p-value cutoff of 0.05 and an odds ratio ≥ 2. Functional groups
246 were defined based on published genome annotations (Rispe et al., 2020).

247

248 Gene co-expression network analysis

249 Genes with 0.5 counts per million (CPM; about 10 raw gene counts) in at least
250 four samples were included for network construction using WGCNA (Langfelder &
251 Horvath, 2008). Gene count matrices were normalized using DESeq2's vst (variance
252 stabilizing transformation) function (v. 1.42.1). A signed gene co-expression network
253 was constructed based on hierarchical clustering of dissimilarity in gene connectivity (1-
254 TOM) with the soft power parameter (β) set to 16 to ensure the network had
255 approximate scale-free topology. Genes were assigned to modules using the
256 cutreeDynamic function with minimum module size = 20 and module detection
257 sensitivity (deepSplit) = 2. Heat maps of module eigengenes and normalized gene
258 expression values (z-scores of vst-transformed count data) were made using pheatmap
259 v. 1.0.12 (https://github.com/raivokolde/pheatmap; rows were clustered based on
260 Euclidean distance. Correlations between *D. vitifoliae* fitness and gene co-expression
261 modules were tested by linear regression of average fitness on each host genotype and
262 average module eigengene expression using the lm function in the R stats package v.
263 4.3.1 where mean fitness = $\beta 0 + \beta 1 \times$ mean eigengene + ϵ. Scatter plots of eigengene-
264 fitness correlations were printed with results from the linear regression test using
265 ggplot2 v. 3.5.0 (https://ggplot2.tidyverse.org/). Gene co-expression modules were
266 tested for overrepresentation/enrichment of gene functional groups based on Fisher's
267 exact tests as described above.
268
269 <u>KEGG term enrichment analysis</u>
270 eggNOG-mapper v. 2 (Cantalapiedra et al., 2021) was used to assign KEGG
271 terms to the predicted proteome of *D. vitifoliae* based on precomputed orthology
272 assignments. eggNOG-mapper assigned KEGG terms to 7,532 proteins out of 15,076
273 queried. To test for KEGG term enrichment for subsets of genes (e.g. differentially
274 expressed genes vs all KEGG-annotated genes) we performed hypergeometric over-
275 representation analysis using the 'enrich' function of the R package clusterProfiler v.
276 4.10.1 (G. Yu et al., 2012). KEGG terms were considered significantly enriched based
277 on a BH-adjusted p-value cutoff of 0.05 and a gene count ≥4.
278
279 <u>Multiple Sequence Alignment and phylogenetic analysis</u>

280 To determine sequence similarity and phylogenetic relationships of *D. vitifoliae*
281 ARabinoGalactan-Like (ARGL) proteins and putative homologs (based on BLASTp
282 searches), we performed Multiple Sequence Alignment (MSA) using the Clustal Omega
283 web application (https://www.ebi.ac.uk/jdispatcher/msa/clustalo; Sievers & Higgins,
284 2018). Alignment results were visualized in a sequence logo plot using the WebLogo
285 web application (https://weblogo.threeplusone.com; Crooks et al., 2004). Phylogenetic
286 trees were constructed from the Clustal Omega MSA output using the Interactive Tree
287 of Life (iTOL) v. 6 web application (https://itol.embl.de; Letunic & Bork, 2024).
288
289 Whole-genome bisulfite sequencing
290 For WGBS, *D. vitifoliae* 'G0' adults and 'G0' and 'G1' eggs were collected from
291 leaf galls at 15-23 DPI and kept at -80 °C until DNA extraction. For 'G1', DNA was
292 isolated from pooled eggs collected from 'G1' galls, i.e. progeny of the 'generation 1'
293 adults used for RNA-seq. Galls from one plant were pooled together to make one
294 biological replicate. DNA was extracted from 200-250 eggs or 15-20 adults per replicate
295 using a CTAB protocol based on the method described by Mugford et al. (2020). The
296 quality and quantity of DNA was tested by running it on a 2% agarose gel and
297 measurement with a Qubit Fluorometer. We prepared 150 bp paired-end WGBS
298 libraries and used Illumina NovaSeq at the UC Davis Genome Center
299 (https://dnatech.ucdavis.edu/#C2). Library prep and sequencing for the 'G0' eggs and
300 adults was performed similarly by Novogene using Illumina NovaSeq
301 (https://www.novogene.com/us-en/services/research-services/epigenome-
302 sequencing/whole-genome-bisulfite-sequencing/). On average, we generated 47.2 (±
303 1.20 s.d.) million paired-end reads per sample (Table S1). Sequencing data were
304 uploaded to the NCBI Sequence Read Archive (SRA) repository under Bioproject
305 accession PRJNA1211173.
306
307 DNA methylation analysis
308 Read and base quality statistics were generated for raw and processed WGBS
309 reads using fastqc (version 0.11.9) (https://github.com/s-andrews/FastQC). Raw reads
310 were initially trimmed and filtered using Trim Galore (version 0.6.7)

311 (https://github.com/FelixKrueger/TrimGalore) with default parameters plus an 80 bp
312 minimal read length filter. Fastqc results showed the 5' and 3' ends of reads had
313 artifactual base sequences that were not removed by quality trimming, and libraries had
314 poor alignment efficiency when mapped to the bisulfite-converted reference genome
315 (Rispe et al., 2020). Thus, 25 bp at the 5' ends and 20 bp at the 3' ends were trimmed
316 from all reads using Trim Galore. Reads were mapped to the reference genome using
317 bismark v. 0.24.1 (Krueger & Andrews, 2011). On average, 59.4 % (± 0.71 s.d) of reads
318 uniquely mapped to the bisulfite-converted reference genome (Table S1). After
319 removing PCR duplicate reads, the average coverage was 5.21 Gigabases (± 1.64 s.d),
320 or 17.7x (± 5.59 s.d) genome coverage (Table S1). Genome-wide CpG, CHG, and CHH
321 methylation percentages for each sample were extracted from the bismark alignment
322 report and plotted using ggplot2.
323 To determine which CpG sites were significantly methylated, a binomial test was
324 performed using the bisulfite conversion (C to T) error rate, which was 0.3% (± 0 s.d,
325 n=4) based on the CpG methylation percentage of spiked-in lambda bacteriophage
326 DNA. CpG sites were filtered based on a BH-adjusted p-value cutoff of 0.001, which
327 removed CpG sites that had less than ~10% methylation (methylated reads divided by
328 all aligned reads). CpG sites were annotated with gene information (gene ID, functional
329 group) and genome features (UTRs, introns, exons, intergenic) using the 'intersect'
330 command of bedtools v. 2.30.0 (Quinlan & Hall, 2010). To assess global differences in
331 CpG methylation between samples, principal component analysis (PCA) was performed
332 using prcomp (R stats package v. 4.3.1). For PCA, we included only CpG sites that
333 were covered by ≥4 reads in all samples, were methylated in at least one sample
334 (binomial test, BH-adjusted p-value cutoff of 0.001), and had non-zero variance. To
335 determine if any *D. vitifoliae* genes were differentially methylated after feeding on
336 different hosts, we used a logistic regression with a F-test and applied a q-value cutoff
337 of 0.05 and a ≥10% methylation difference cutoff using the R package methylKit v.
338 1.28.0 (Akalin et al., 2012). The 'intersect' command of bedtools v. 2.30.0 was used to
339 determine if any differentially methylated genes were also differentially expressed (i.e.
340 host-responsive).

341 Additionally, we wanted to understand how gene expression plasticity correlated
342 with DNA methylation. We used all host-by-host contrasts to calculate the mean
343 absolute value of log2-fold-change in gene expression as a measure of transcriptional
344 plasticity. The 'G0' (starting colony) adult samples were used to calculate DNA
345 methylation levels for each gene, since these were sampled at the same developmental
346 stage (adults) as our RNA-seq data. Transcriptional plasticity and DNA methylation
347 percentage (% reads with methylated cytosines divided by total aligned reads) was
348 plotted for each gene in a scatterplot and a LOESS (Locally Estimated Scatterplot
349 Smoothing) line was fitted to the data to visualize the trend. We then tested for
350 correlation between transcriptional plasticity and DNA methylation with a Spearman
351 rank correlation test using the R stats package v. 4.3.1. Lastly, genes were binned into
352 quartiles based on the mean absolute value of log2-fold-change, then pairwise Wilcoxon
353 rank-sum tests were performed to test for significant differences in DNA methylation
354 levels between quartiles.
355 All scripts for data processing and bioinformatic analyses are available online at
356 https://github.com/wcoll010/Grape-phylloxera-phenotypic-plasticity-during-host-transfer.
357
358 **Results**
359 Insect fitness differs significantly across host grapevine genotypes
360 To determine how fitness differs across grapevine hosts, we experimentally
361 introduced a clonal line of *D. vitifoliae* to a panel of grapevine genotypes, including a
362 non-native host (Cab33) and interspecific hybrids of native North American grapevine
363 species (Figure 1). Fitness varied across host grapevine genotypes (Figure 1A) but the
364 grapevine genotypes SW (*V. riparia* X *V. rupestris*), 5BB (*V. riparia* X *V. berlandieri*),
365 and 3309 (*V. riparia* X *V. rupestris*) were incompatible, i.e. fully resistant to colonization.
366 Attempted feeding by *D. vitifoliae* induced small necrotic lesions on leaves of genotypes
367 SW and 5BB within six days after experimental infestation (Figure 1D and 1E).
368 Interestingly, genotype 3309 showed symptomless resistance after six days (Figure 1F)
369 but eventually showed necrotic lesions in leaves at feeding sites three weeks after
370 infestation (Figure S1). Fitness was lower on both Cab33 and SO4 (*V. berlandieri x V.*

371 *riparia*) compared to the other three susceptible hosts (Figure 1G). All other pairwise
372 comparisons between host genotypes revealed no differences in *D. vitifoliae* fitness.
373
374 *D. vitifoliae* gene expression is highly dynamic and responsive to host grapevine
375 genotype
376 To understand the genome-wide variation in *D. vitifoliae* gene expression
377 induced upon transfer to different host grapevine genotypes, we isolated RNA from *D.*
378 *vitifoliae* adults after feeding on new hosts for a single generation and performed RNA-
379 seq. Clustering of samples based on multidimensional scaling (MDS) indicated host
380 environment induces distinct changes in *D. vitifoliae* gene expression (Figure 1H).
381 Differential gene expression analysis revealed 3,706 were host-responsive (1.5-fold-
382 change in any pairwise comparison, BH-adjusted $p < 0.05$; Table 1), which was 28% of
383 the 13,125 total expressed genes. We then performed a Fisher's exact test to identify
384 gene functional groups that are enriched (over-represented) among the host-responsive
385 genes relative to the genome-wide background, which included all 25,808 *D. vitifoliae*
386 gene models. Genes encoding predicted secretory effectors (adj. p = 1.7e-127, odds
387 ratio = 3.30), developmental regulators (adj. p = 1.6e-14, odds ratio = 4.55), cuticle- and
388 chitin-associated proteins (adj. p = 1.5e-06, odds ratio = 3.03), detoxification enzymes
389 (adj. p = 1.9e-05, odds ratio = 2.14), and proteins involved in general metabolism (adj. p
390 = 8.9e-03, odds ratio = 5.23) were enriched among host-responsive genes (Table 1).
391 Pair-wise analysis of gene differential expression (DE) revealed that the non-
392 native host Cab33 induced the strongest change in gene expression compared to other
393 hosts; 3,432 (92.6%) of the total 3,706 host-responsive genes show differential
394 expression in contrasts between Cab33 and the other four host genotypes (Figure 2A).
395 Only 19 genes were differentially expressed in all contrasts with Cab33 (Figure 2B); 6 of
396 these genes encoded predicted secretory effectors, including 3 RING-type E3 ligases
397 (DV3020556, DV3022321, and DV3025927). We further investigated the types of genes
398 that are up- and down-regulated when *D. vitifoliae* feeds on Cab33 using gene
399 enrichment analysis. Of the host-responsive genes, 2,667 (72%) were upregulated
400 during interaction with Cab33 relative to all other hosts. Genes encoding predicted
401 effectors, cuticle- and chitin-associated proteins, and detoxication enzymes were

402 enriched among genes upregulated on Cab33 (Table S2). Enriched KEGG terms for
403 upregulated genes included glutamate receptors, fatty acyl-CoA reductases, cathepsin
404 B cysteine proteases, nicotinic acetylcholine receptors, and organic acid transporters
405 (Figure 2C). Genes downregulated during interaction with Cab33 are enriched for
406 functions related to development (Fisher's exact test, BH-adjusted p = 1.59e-09, odds
407 ratio = 6.36) and KEGG terms for lactose galactohydrolase/β-glucosidases (PFAM
408 domain GH1) and serine protease inhibitors (Figure 2D). Overall, these results suggest
409 that the *D. vitifoliae* transcriptome is highly plastic, especially at genes encoding
410 secretory proteins including putative effectors and genes involved with response to and
411 protection against environmental stress.
412
413 Four gene co-expression modules co-vary with *D. vitifoliae* fitness
414 To identify groups of genes that likely function together in shared biological
415 processes, we performed weighted gene co-expression network analysis using 13,917
416 genes after removing 'zero genes' that showed negligible expression. A signed gene co-
417 expression network was constructed that contained 88 modules based on hierarchical
418 clustering of gene connectivity dissimilarity (Figure 3A and 3B). Gene co-expression
419 modules were named based on colors randomly assigned by the WGCNA program
420 (Langfelder & Horvath, 2008). To identify groups of co-regulated genes (modules) that
421 might underlie differences in *D. vitifoliae* fitness across host genotypes, we tested for
422 correlation between module eigengenes (first principal component of the module and
423 the most representative gene) and fitness using linear regression tests. Five
424 eigengenes significantly correlated with *D. vitifoliae* fitness (Figure 3C-F); we excluded
425 the 'grey' module from further analysis since it is a bin for genes that cannot be
426 assigned to co-expression modules and are unlikely to be co-regulated.
427 We performed Fisher's exact tests to identify gene co-expression modules that
428 were enriched for host-responsive genes. Twenty-one out of the 88 modules were
429 enriched for host-responsive genes (odd ratio >2, BH-adjusted p <0.05) (Table S3),
430 which reflects the high number of transcriptionally plastic genes in the dataset. The
431 'darkorange2' module was both enriched for host-responsive genes and negatively
432 correlated with *D. vitifoliae* fitness (Figure 3E). Based on KEGG term enrichment

433 analysis, ‘darkorange2’ is overrepresented for genes that encode glycoside hydrolase
434 family 1 enzymes (PFAM GH1), and show distinct patterns of expression across host
435 genotypes (Figure S3). A small cluster of 24 genes is highly expressed during
436 interactions with native hosts 101-14 and 1103 and decreases in expression during
437 interactions with Cab33 and SO4, which are hosts that maintain relatively low *D.*
438 *vitifoliae* fitness. The remaining 66 genes in the module are grouped into a larger cluster
439 that shows the exact opposite trend in expression, largely increasing in expression
440 during interaction with Cab33 and SO4 relative to 101-14 and 1103. These results show
441 the ‘darkorange2’ module is highly responsive to host environment and may contribute
442 to fitness variation in *D. vitifoliae* during interaction with non-optimal hosts.
443
444 <u>One module contains a cluster of conserved and proline-rich secretory proteins</u>
445 The ‘thistle’ module was most strongly correlated with *D. vitifoliae* fitness (R^2 =
446 0.94, p = 0.005; Figure 3C-F) and enriched with genes encoding predicted secretory
447 effectors (Fisher’s exact test, BH-adjusted p = 0.028, odds ratio = 3.32). Other than the
448 11 predicted effectors, the remaining 28 genes in this module did not belong to any
449 predicted functional group. Four predicted effectors were homologous to *Acyrthosiphon*
450 *pisum* ‘lysine-rich arabinogalactan protein 19-like’ (accession XP_001948476.1), based
451 on previously published BLASTp alignments (Rispe et al., 2020). We termed these
452 genes as encoding ‘<u>AR</u>abino<u>G</u>alactan-<u>L</u>ike’ (ARGL) proteins. *ARGL* genes show highly
453 similar patterns of transcript expression across treatments, generally increasing in
454 expression during interaction with high-fitness native hosts 101-14, 1103, and 99R
455 relative to Cab33 (Figure 4B). We also noticed that the *ARGL* genes are located in a
456 tandem cluster in the genome on scaffold 40 (Figure 4A). Within this same ~50-kb
457 spatial cluster are two more predicted effector-encoding genes (DV3003881 and
458 DV3003883) and three ‘null’ genes (DV3003873, DV3003874, DV3003879); two of the
459 three null genes (DV3003873 and DV3003874) are also assigned to the ‘thistle’ module,
460 suggesting they are co-regulated with *ARGL* genes. To determine if *ARGL* genes have
461 highly similar sequences and shared protein motifs, we performed Multiple Sequence
462 Alignment (MSA) of the seven ARGL proteins using Clustal Omega (Sievers & Higgins,
463 2018). A sequence logo plot of the ARGL protein MSA showed that the proteins have a

464 high proportion of proline residues and are highly conserved, especially at proline
465 residues (Figure S4). We then constructed phylogenetic trees from the MSA of protein
466 sequences and coding sequences (CDS) for all 2,743 predicted secretory effector
467 proteins and found *ARGL* coding sequences clustered together into a monophyletic
468 group (Figure S5), suggesting they may be members of a gene family that evolved
469 through tandem duplication. Despite being highly conserved at the amino acid level and
470 clustered together based on coding sequences, ARGL protein sequences did not cluster
471 together in a monophyletic group (Figure S6). Thus, ARGL proteins may have some
472 structural and functional divergence from one another.
473 To identify putative orthologs of *ARGL* genes, we performed a BLASTp search
474 using the *ARGL* gene DV3003875 queried against the NCBI non-redundant protein
475 sequences (nr) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). We
476 then performed reciprocal BLASTp alignments of the top 20 BLASTp hits against the *D.*
477 *vitifoliae* genome. Seventeen out of the 20 proteins produced reciprocal best hits for the
478 DV3003875 *ARGL* gene. We then investigated the evolution of the *ARGL* locus by
479 performing MSA of the seven ARGL proteins with the 17 putative orthologs of ARGL
480 DV3003875, which spanned adelgids (*Adelges cooleyi*) and eight aphid species (Table
481 S4). Remarkably, a roughly 200-amino-acid region of all 27 proteins is highly
482 conserved, especially at proline residues (Figure 4C and Figure S7). We also searched
483 the proteins for predicted functional domains using InterPro
484 (https://www.ebi.ac.uk/interpro/search/sequence/). Notably, all 24 proteins contain
485 predicted secretory signal peptides but generally lack any known functional domains,
486 with two exceptions. The *Aphis glycines* 'hypothetical protein AGLY_000967' (accession
487 KAE9545424.1) and the *Metopolophium dirhodum* 'uncharacterized protein
488 LOC132951494' (accession XP_060879307.1) contain the InterPro-unintegrated motif
489 (PRINT entry) PR01217 (Proline-rich extensin signature). Extensin proteins are notably
490 characterized as cell wall-associated proteins in plants (Moussu & Ingram, 2023),
491 although whether the PR01217 motif confers any similar function to plant extensin
492 proteins is unclear. Overall, our analysis of the 'thistle' co-expression module revealed
493 an under-described group of genes encoding predicted secretory proteins, that are
494 tightly co-regulated and positively correlated with fitness at the transcript level. ARGL

495 proteins also are proline-rich, highly conserved, and belong to a putative family of
496 secretory proteins seemingly widespread in the Aphidomorpha infraorder of Hemipteran
497 insects. Together, these results suggest *ARGL* genes play an important role in host use
498 in aphidomorph insects.
499
500 DNA methylation in *D. vitifoliae* is mostly stable
501 In addition to our analysis of the *D. vitifoliae* transcriptome, we analyzed genome-
502 wide patterns of DNA methylation using WGBS. Our goal was to first understand
503 patterns of DNA methylation in the *D. vitifoliae* epigenome and then to determine
504 whether host genotype induced changes in DNA methylation after insects fed on
505 different hosts for a single generation. DNA methylation in *D. vitifoliae* occurs
506 exclusively in the CpG context, and about 3.5-4% of CpG sites are methylated (Figure
507 5A), which is similar to what is reported in other Hemipteran insects (Bewick et al.,
508 2016; Mathers et al., 2019). CHH and CHG methylation rates were around 0.3%, which
509 is equal to the false positive rate. As in other insects, DNA methylation in *D. vitifoliae*
510 occurs mostly in exons and other gene body regions (Figure 5B).
511 To determine variation in DNA methylation across our samples at a global level,
512 principal component analysis (PCA) was performed using the 201,448 CpG sites that
513 were methylated in at least one sample and had non-zero variance. Our PCA revealed
514 no distinct clustering based on treatment group (host grapevine genotype) except for
515 the adult samples (Figure 5C). We repeated the PCA twice: once after removing the two
516 potential outlier samples, and then once more after further removing the adult samples.
517 The results similarly showed no distinct clustering based on host genotype (Figure S8).
518 We additionally tested whether any genes were differentially methylated between
519 insects reared on different host grapevine genotypes using methylKit (Akalin et al.,
520 2012). Eight genes were host-responsive at the DNA methylation level (Table S5), but
521 none of these genes were differentially expressed at the transcript level. All 3,706 genes
522 that were transcriptionally plastic (host-responsive) showed no significant change in
523 DNA methylation in the subsequent generation. These results show that DNA
524 methylation in *D. vitifoliae* varies across developmental stages (adults compared to
525 eggs) but is mostly not responsive to host environment. We also found that host-

526 induced variation in gene expression does not lead to any significant change in gene
527 body methylation in progeny.
528
529 Gene transcriptional plasticity is negatively correlated with DNA methylation
530 We wanted to further understand how host-induced transcriptional plasticity
531 correlates with DNA methylation in *D. vitifoliae*. We hypothesized that transcriptionally
532 plastic (host-responsive) genes lack DNA methylation, which might explain why host-
533 responsive genes acquired no significant changes in DNA methylation. This hypothesis
534 is supported by other data showing that DNA methylation in insects occurs primarily in
535 gene body regions of stably expressed genes (Glastad et al., 2016; Libbrecht et al.,
536 2016; Mathers et al., 2019). To quantify the degree of transcriptional plasticity for genes,
537 mean absolute values of log2 fold changes in gene expression across all host-by-host
538 contrasts were calculated. Gene-level transcriptional plasticity and DNA methylation
539 level was plotted for genes that were expressed and had CpG methylation data (n =
540 12,368) (Figure S9). There was a significant negative correlation between
541 transcriptional plasticity and DNA methylation (Spearman's ρ = -0.54, $p < 2.2e-16$). To
542 further assess correlation between gene expression plasticity and DNA methylation,
543 genes were binned into quartiles based on transcriptional plasticity and pair-wise
544 Wilcoxon rank-sum tests were performed. This also showed a negative correlation
545 between transcriptional plasticity and DNA methylation, with transcriptionally plastic
546 genes having significantly less methylation compared to stably expressed genes (Figure
547 5D). These data indicate gene body DNA methylation contributes to gene expression
548 regulation in *D. vitifoliae* and possibly stabilize transcriptional plasticity when *D. vitifoliae*
549 faces new host environments.
550
551 **Discussion**
552 The transcriptional and epigenetic mechanisms that enable parasites and
553 pathogens to colonize of new hosts remain only partially understood. In this study, we
554 found the transcriptome of the specialist insect *D. vitifoliae* is remarkably plastic, but
555 more so when colonizing a novel (non-native) host compared to native hosts. The
556 transcriptionally plastic genes that increased in expression were enriched for predicted

557 secretory effectors, detoxification enzymes, and cuticle- and chitin-associated proteins –
558 genes linked to the herbivore lifestyle (Karban, 2020; Peláez et al., 2023). We found
559 gene co-expression modules correlated with fitness, suggesting plasticity in
560 transcriptional networks contributes to *D. vitifoliae* adaptation to host plants. In contrast,
561 DNA methylation did not respond to host environment but negatively correlated with
562 transcriptional plasticity. These results highlight that rapid transcriptional change may
563 enable host shifts in a specialist herbivore similar to how polyphagous herbivores adapt
564 to feed on divergent hosts (Dermauw et al., 2013; Mathers et al., 2017; Snoeck et al.,
565 2018). These results also link a lack of DNA methylation to increasingly plastic genes
566 and suggest gene body methylation plays an important role in constitutive gene
567 expression during colonization of new hosts. Together these results help us understand
568 how transcriptional and epigenomic variation enable adaptation to novel hosts by
569 specialist herbivores.
570 Because *D. vitifoliae* is a specialist herbivore that evolved to feed on a narrow
571 range of hosts in the *Vitis* genus, we predicted that transcriptional plasticity induced by
572 transfer to different compatible hosts would be low; this has been shown for other
573 specialist insect species in similar experiments (Govind et al., 2010; Q. Yu et al., 2016;
574 Roy et al., 2016; Birnbaum et al., 2017; Schweizer et al., 2017). Contrary to this
575 prediction, the *D. vitifoliae* transcriptome was highly plastic. Of all expressed genes,
576 3,706 (28%) were host responsive (Table 1), and the majority of these were upregulated
577 while feeding on the non-native host European wine grape *V. vinifera* (cv Cab33) (Table
578 S2). Predicted detoxification genes were enriched among genes upregulated during
579 interaction with *V. vinifera*, and included numerous cytochrome P450s, glutathione S-
580 transferases and UDP-glycosyltransferases, and ABC transporters. These patterns
581 suggest that all three phases of xenobiotic detoxification are reprogrammed when *D.*
582 *vitifoliae* feeds on *V. vinifera* (Heidel-Fischer & Vogel, 2015), likely helping to overcome
583 novel host defenses. Predicted effectors were also enriched among genes upregulated
584 while feeding on *V. vinifera* (Table S2). This functional group includes over 2,700
585 secretory proteins with effector attributes, including putative effectors known to interact
586 with host grapevine proteins such as RING-type E3 ligases (Zhao et al., 2019).
587 Interestingly, three RING-type E3 ligases were consistently upregulated when *D.*

588 *vitifoliae* fed on the non-native host *V. vinifera* compared to the other hosts, supporting
589 the hypothesis that protein modification is a key pathway to coopt hosts (Zhao et al.,
590 2015; Huang et al., 2021). The subset of genes identified here serve as targets for
591 further functional characterization to validate mechanisms of host adaptation in *D.*
592 *vitifoliae* and improve management of grape against *D. vitifoliae.* More broadly, these
593 results show that at least some lineages of specialist herbivores, despite long-term
594 ecological specialization, rely on substantial transcriptional plasticity for ecological
595 persistence, contrary to predictions (Sexton et al., 2017; Birnbaum & Abbot, 2020).
596 Our analysis of KEGG terms similarly revealed that genes putatively involved
597 with insect adaptations were overrepresented among genes upregulated while feeding
598 on the non-native host *V. vinifera*. These genes included ionotropic glutamate receptors
599 which can function as chemosensory receptors (Benton et al., 2009; Croset et al.,
600 2010), fatty acyl-CoA reductases (FAR) which can modify cuticle composition (Li et al.,
601 2020; Di et al., 2023), and cathepsin B cysteine proteases (Figure 2C). Notably, aphid
602 cathepsin B proteases function as secretory effectors during interaction with divergent
603 host plants (Mathers et al., 2017). Other recent studies show *M. persicae* cathepsin B
604 genes are expressed in response to abiotic stresses and important for thermotolerance
605 (Liu et al., 2023; Zhang et al., 2025). Thus, cathepsin B proteins in related aphids
606 contribute to adaptation to different environments and may also underlie host adaptation
607 in *D. vitifoliae*. In summary, we found many of the genes that increase in expression
608 during colonization of a non-native host are putatively involved with key adaptations in
609 herbivorous insects – detoxification of plant defense metabolites, direct manipulation of
610 hosts via effector protein secretion, and cuticle modification – highlighting that specialist
611 lineages dramatically reprogram critical pathways to colonize new environments.
612 In addition to differential expression analysis, we wanted to understand how
613 transcriptional plasticity mediates host use by correlating clusters of co-expressed
614 genes (gene co-expression modules) with variation in *D. vitifoliae* fitness across
615 different hosts. Four gene co-expression modules were significantly correlated with
616 variation in fitness, indicating that plasticity in transcriptional networks can contribute to
617 host plant adaptation (Figure 3). The ‘thistle’ co-expression module most strongly
618 correlated with fitness (Figure 3C) and was enriched with genes coding for predicted

619 secretory effector proteins (Fisher's exact test, BH-adjusted p = 0.028), while the
620 remaining genes in the module were not assigned to any predicted functional group.
621 This module also contained several co-regulated genes that are organized as a spatial
622 cluster in the genome, encode predicted secretory effector proteins (ARGL; Figure 4),
623 and are shared among aphids indicating their likely presence in the common ancestor of
624 the Aphidomorpha lineage. *D. vitifoliae* ARGL proteins and putative ARGL orthologs
625 show a high degree of amino acid conservation and retained secretory function over the
626 course of more than 160 million years of evolution (Figure 4C and Table S4)(Ren et al.,
627 2013), highlighting an important role for ARGL proteins in Aphidomorph biology or
628 ecology. Some of the putative ARGL orthologs in aphids and adelgids (*A. cooleyi*)
629 contain predicted proline-rich extensin motifs, and other putative ARGL orthologs are
630 annotated as 'lysine-rich arabinogalactan protein 19-like', 'vegetative cell wall protein
631 gp1-like', and 'leucine-rich repeat extensin-like protein 1' (Table S4). Although the
632 function of insect ARGL proteins remains unclear, a common theme among annotations
633 is the sensing and maintenance of plant cell walls (Herger et al., 2019; Ellis et al., 2010;
634 Ferris et al., 2001). As a central regulator of cellular structure and immune signaling in
635 plants, the plant cell wall is a critical interface for herbivore-host interaction (Wolf, 2022;
636 Erb & Reymond, 2019), and thus a likely target of insect secretory effectors. Whether
637 ARGL proteins play a role in host cell wall manipulation or host interaction more broadly
638 requires further functional genetic analysis, although our computational analyses here
639 suggest they are important for host use across aphidomorph insects.
640 In addition to our analysis of transcriptomic plasticity induced by host transfer, we
641 analyzed DNA methylation in *D. vitifoliae* after host transfer. To our knowledge, no other
642 studies have measured plasticity in both the transcriptome and epigenome of an
643 herbivore during interaction with novel hosts. Thus, we lack data for how host plants
644 shape the epigenome of herbivores and on the correlation between gene expression
645 variation and epigenetic marks. Importantly, our data were collected across two different
646 generations of *D. vitifoliae*. We collected RNA-seq data from adult *D. vitifoliae* that fed
647 on new host plants for one generation and DNA methylation in the progeny eggs, which
648 allowed us to test for correlation between host-induced plasticity in gene expression and
649 variation in DNA methylation in the subsequent generation. Although the *D. vitifoliae*

650 transcriptome was highly plastic across host plants, there was little variation in DNA
651 methylation in the subsequent generation. We found eight genes that were differentially
652 methylated across host plants (Table S5); thus, we did find evidence for host-specific
653 epimutations that could affect host adaptation via change gene expression level
654 (Bonasio et al., 2012; Libbrecht et al., 2016; Mathers et al., 2019), transcript splicing (Li-
655 Byarlay et al., 2013; Glastad et al., 2016), or gene mutation rate (Elango et al., 2009).
656 However, none of these eight genes were differentially expressed across hosts or linked
657 to fitness variation and samples did not cluster based on host genotype (Figure 5C and
658 Figure S8). These patterns show that, unlike the transcriptome, DNA methylation is
659 mostly non-responsive to host plant genotype, and thus unlikely to mediate rapid
660 phenotypic adaptation. We found a significant negative correlation between
661 transcriptional plasticity and DNA methylation, however, with transcriptionally plastic
662 genes being generally depleted in DNA methylation relative to stably expressed genes
663 (Figure 5D and Figure S9). This is consistent with previous findings in other insects
664 (Bonasio et al., 2012; Libbrecht et al., 2016; Mathers et al., 2019). Given the adaptive
665 significance of transcriptional plasticity during host use and host shifts, DNA methylation
666 may play an important role by limiting plasticity of stably expressed genes for which
667 environment-induced plasticity is maladaptive.
668 Taken together, our results suggest that specialist herbivores, despite long-term
669 ecological specialization, can rapidly reprogram their transcriptomes to invade new
670 hosts, and that gene body methylation contributes to the regulation of host-induced
671 transcriptional plasticity. In this example, the switch to the novel host *V. vinifera* comes
672 at a fitness cost but *D. vitifoliae* still persists, highlighting a mechanism for adaptation to
673 increased host breadth that may occur with invasions and range expansions. More
674 broadly, these data provide new evidence for the epigenetic basis of host-induced
675 transcriptional plasticity and can serve as the basis for future functional genetic work to
676 validate genes involved with adaptation to host plants in aphidomorph insects.
677
678
679 **Acknowledgments**

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Author contributions

W.N.C., S.A.Z. and P.D.N. conceived the study. W.N.C. and S.A.Z. performed sample collections and insect performance assays. S.A.Z. performed the DNA and RNA extractions. W.N.C. performed data processing and analysis and wrote the manuscript with advice from S.C.G and P.D.N. All authors read, edited, and approved the manuscript.

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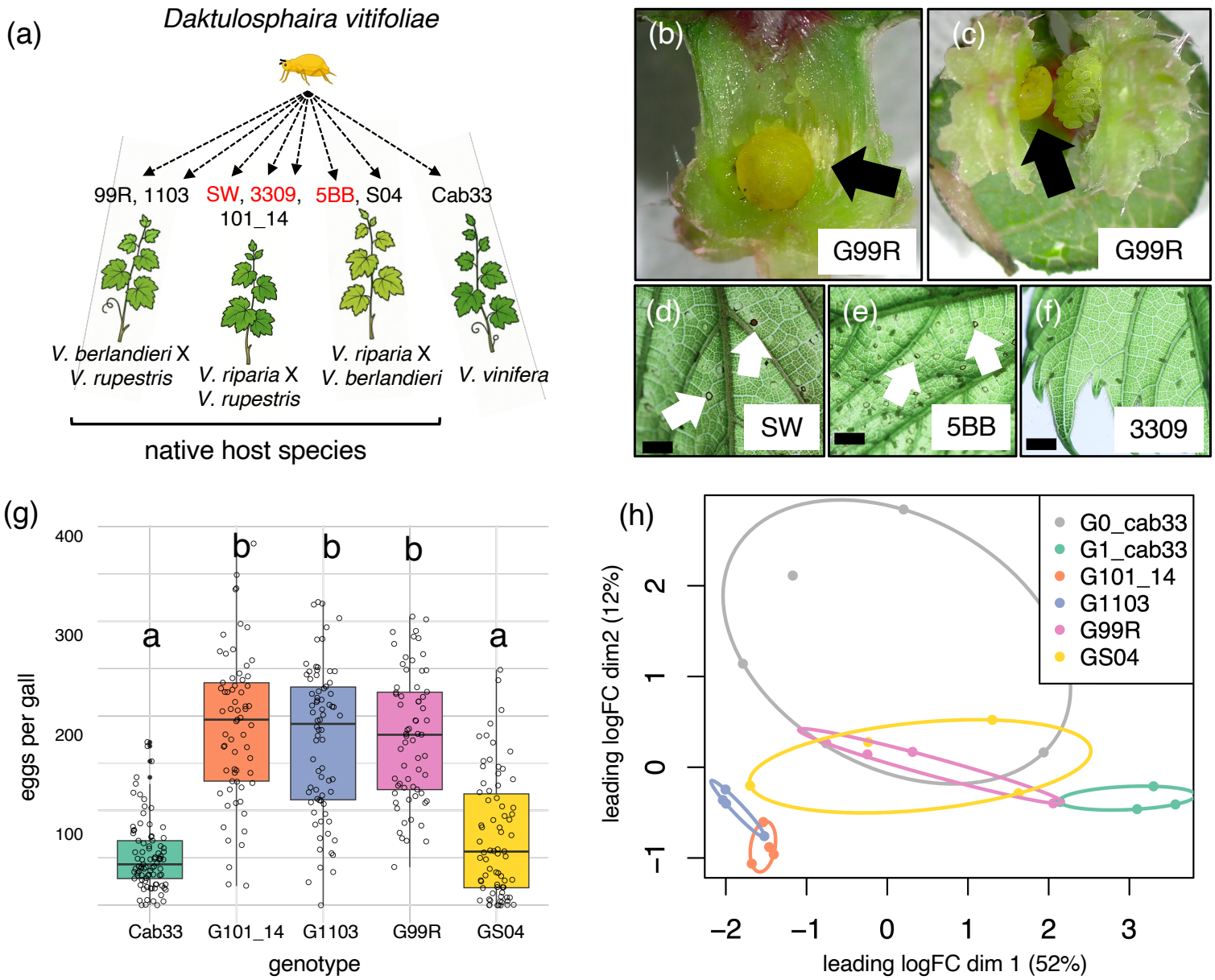
Table 1: Fisher's exact test for functional group enrichment among host-responsive (HR) genes

Annotation Group	BH-adjusted p-value	Odds ratio	# HR genes	Total # genes
General metabolism	8.9E-03	5.23	6	14
Developmental genes	1.6E-14	4.55	53	135
Predicted effectors	1.7E-127	3.30	804	2,743
Cuticle and chitin related proteins	1.5E-06	3.03	35	116
Other	1.4E-33	2.41	319	1,280
Circadian rhythm	not sig.	2.32	4	16
Detoxification of xenobiotics	1.9E-05	2.14	52	222
Epigenetic machinery	not sig.	1.74	1	5
Odorant or gustatory perception	1.9E-05	1.05	14	107
Small RNA machinery	not sig.	0.63	1	12
null	2.5E-74	0.49	2,417	20,998


(a)
Daktulosphaira vitifoliae
99R, 1103
SW, 3309, 101_14
5BB, S04
Cab33
V. berlandieri X V. rupestris
V. riparia X V. rupestris
V. riparia X V. berlandieri
V. vinifera
native host species
(b)
G99R
(c)
G99R
(d)
SW
(e)
5BB
(f)
3309
(g)
eggs per gall
400
300
200
100
a
b
b
b
a
Cab33
G101_14
G1103
G99R
GS04
genotype
(h)
G0_cab33
G1_cab33
G101_14
G1103
G99R
GS04
leading logFC dim2 (12%)
leading logFC dim 1 (52%)
−2
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1057 **Figure 1**: (a) Overview of the initial experimental setup; genotype names in red were resistant to *D. vitifoliae,* insects
1058 died and were excluded from downstream analysis. (b-c) *D. vitifoliae* adult (black arrows) within its gall at 3 weeks after
1059 infestation of host genotype 99R. (d-f) Young leaves of resistant host plants at 6 days after infestation; white arrows
1060 indicate sites of leaf necrosis induced by *D. vitifoliae* feeding/probing; horizontal black scale bars are 1 mm in length.
1061 (g) Box plot showing interquartile range and median (horizontal line within box) of *D. vitifoliae* fitness (eggs per gall)
1062 across susceptible host genotypes; genotypes with letters 'a' are significantly different from those with letter 'b' based
1063 on pair-wise Wilcoxon tests (Benjamini-Hochberg [BH]-adjusted p-values are all less than 0.0001). (h) Multi-
1064 dimensional scaling plot of log-CPM (gene counts per million gene counts) for all RNA-seq samples.
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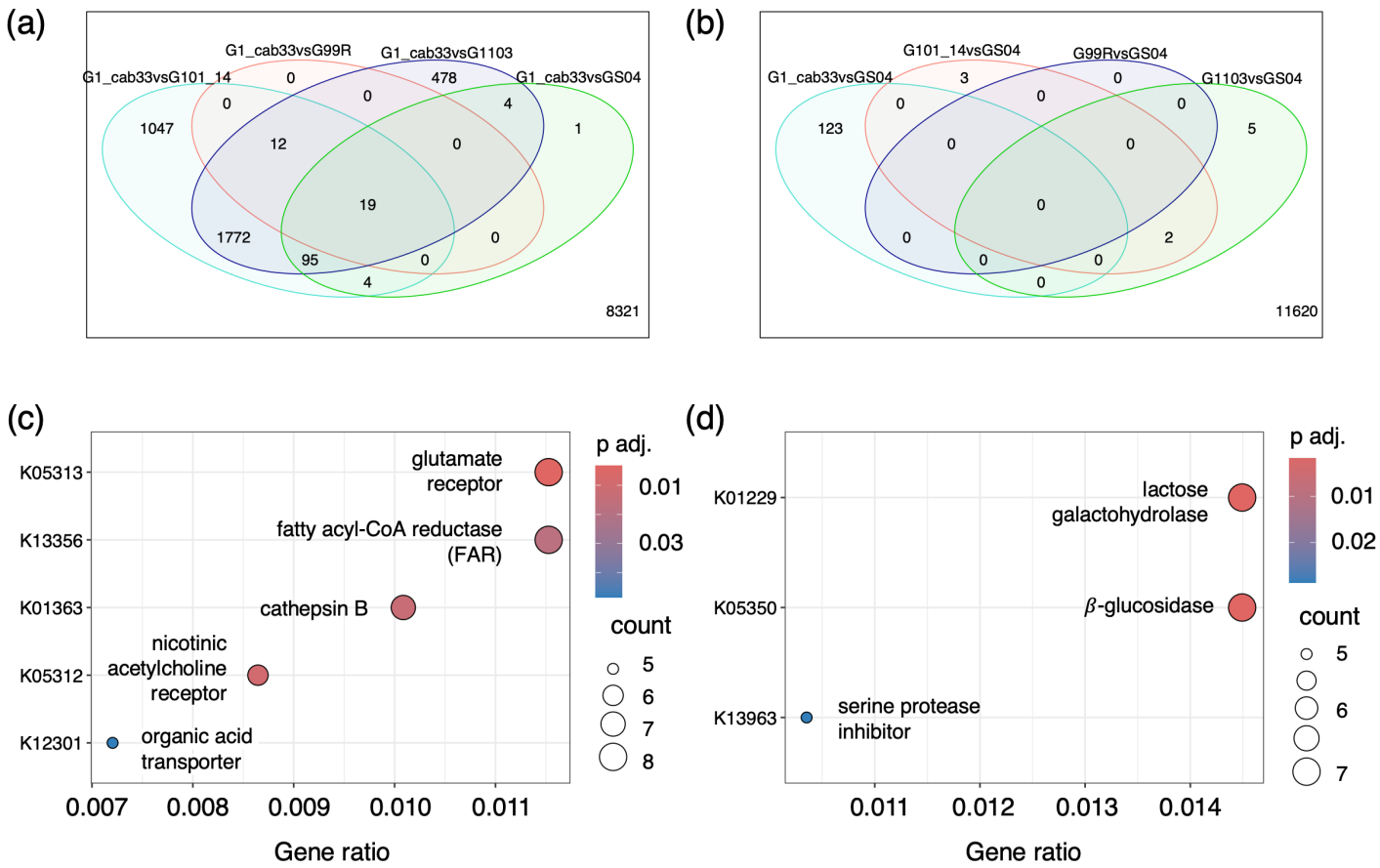

Figure 2: Transcriptional plasticity induced by host transfer. (a-b) Venn diagrams showing the number of differentially expressed *D. vitifoliae* genes contrasting *V. vinifera* cv Cab33 (a) or SO4 (b) with all other hosts genotypes. (c-d) KEGG terms enriched among genes upregulated (c) and downregulated (d) during interaction with *V. vinifera* cv Cab33 compared to all other hosts; colors represent Benjamini-Hochberg (BH) adjusted p-values.

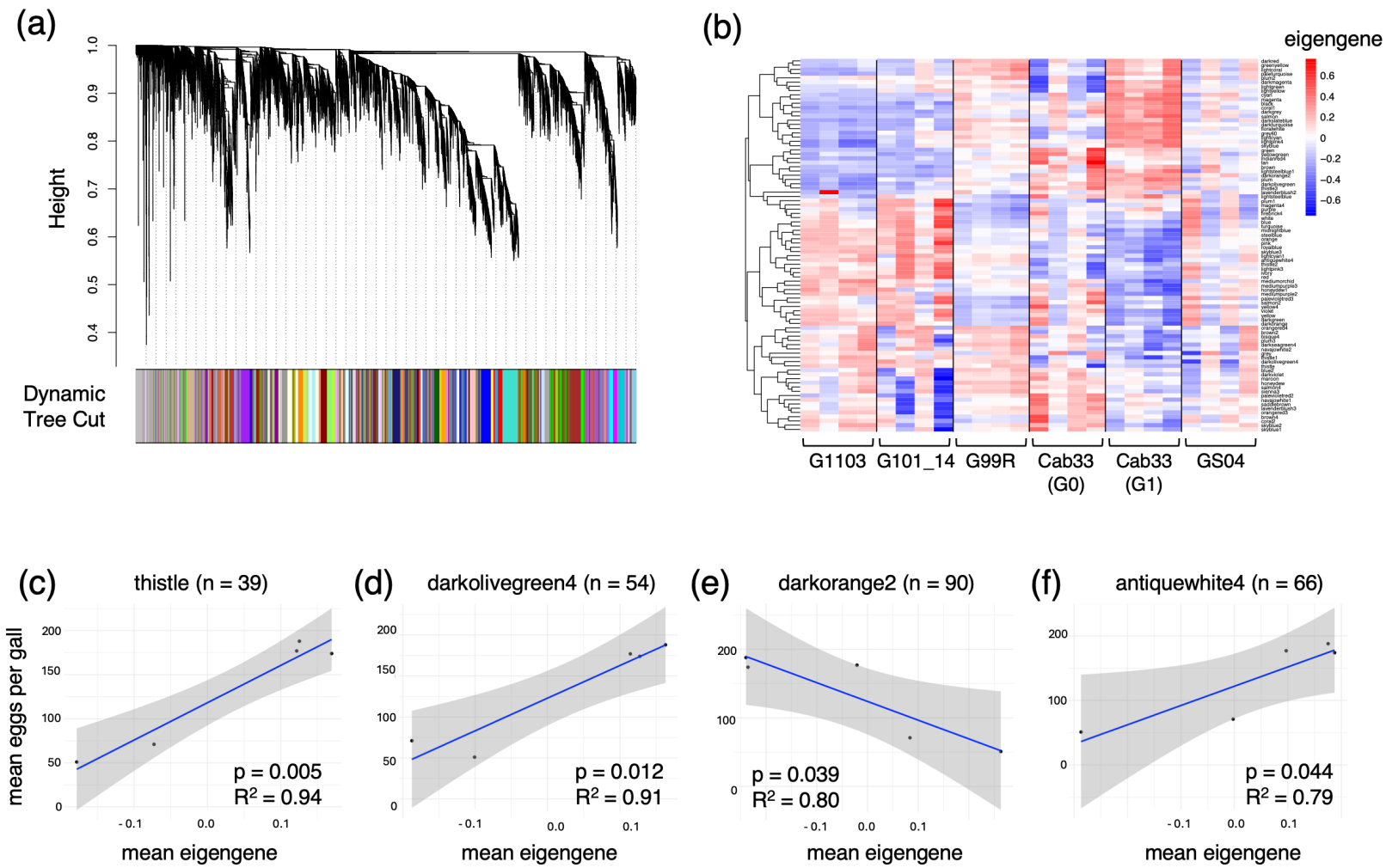

Figure 3: Gene co-expression network analysis. (a) Gene dendrogram for *D. vitifoliae* signed (unidirectional) gene co-expression network and module assignment (bottom). (b) Heatmap of module eigengenes (first principal compenent of the module, the 'most representative' gene) for all 88 modules (verticle axis) clustered based on Euclidean distance. (c-f) Scatter plots and results of linear regression tests for four co-expression modules that correlate in expression with *D. vitifoliae* fitness.

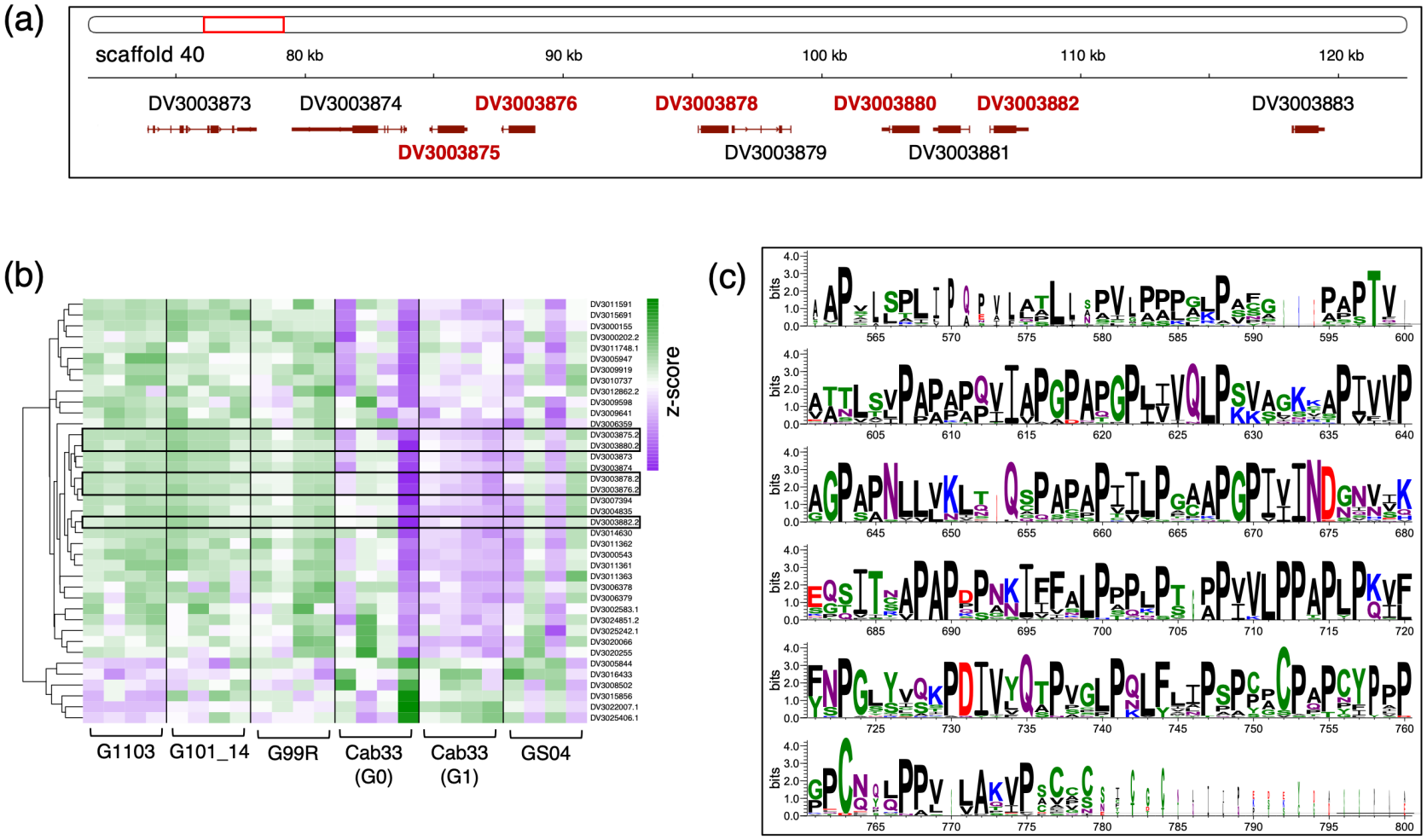

Figure 4: Spatial organization and gene expression profile of ARGL genes and multiple sequence alignment of ARGL proteins. (a) Genome browser view showing the ARGL locus in the *D. vitifoliae* genome; ARGL genes assigned to the thistle gene co-expression module are highlighted with bold red text. (b) Heatmap of normalized gene expression for genes in the thistle module; black rectangles highlight the ARGL genes. (c) Sequence logo plot based on multiple

1092 sequence alignment (MSA) of the seven predicted effector proteins encoded at the ARGL locus and the top seventeen
1093 orthologs of ARGL protein DV3003875; letters represent amino acid residues and letter size denotes the degree of
1094 conservation (larger letters are more conserved).
1095
1096
1097
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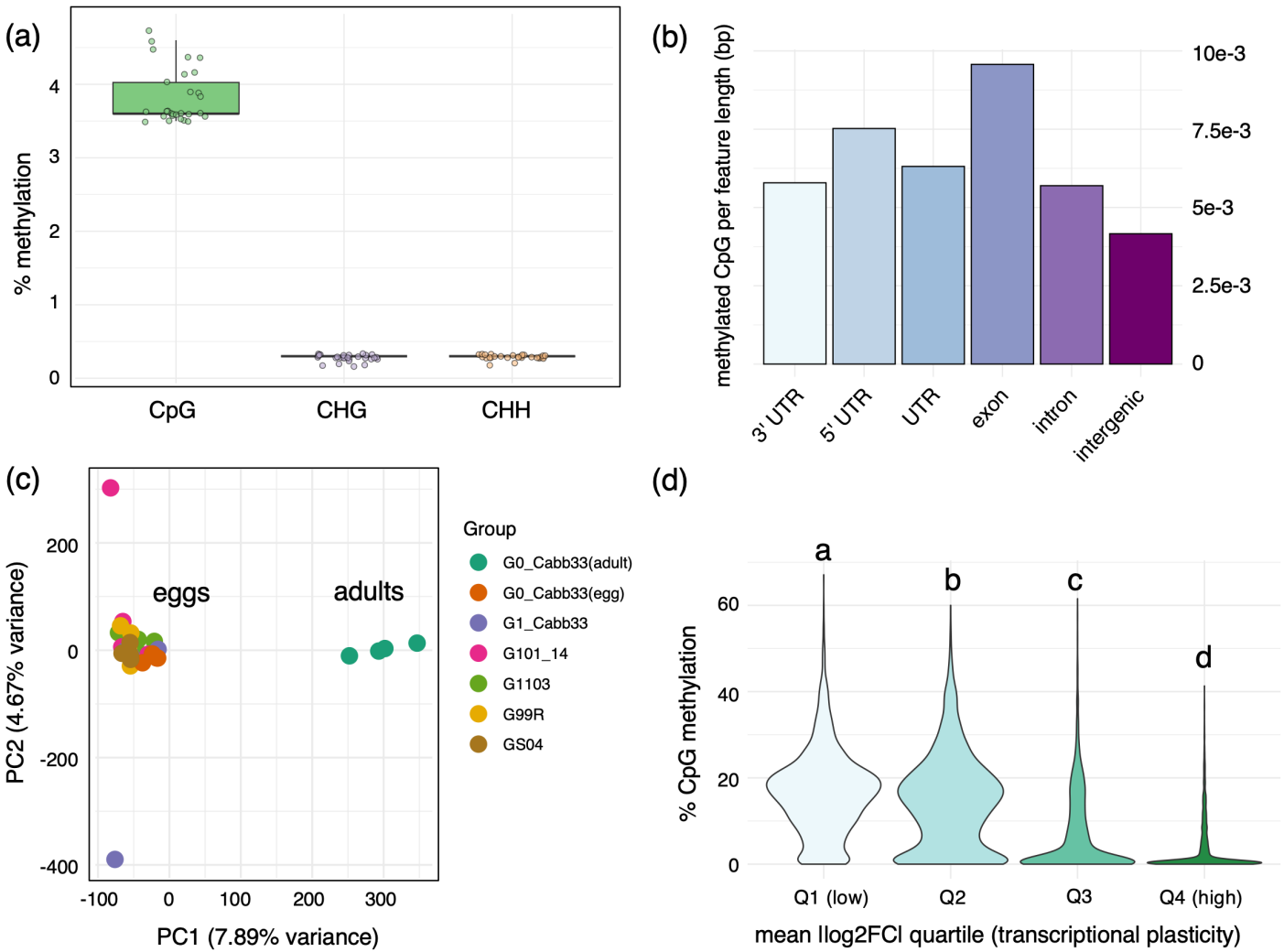

Figure 5: DNA methylation in *D. vitifoliae*. (a) Box plot showing the proportion of methylated cytosines (vertical axis) for each sequence context (CpG, CHG, and CHH). (b) Bar plot showing CpG methylation levels for each genomic region (untranslated regions (UTRs), intron, exon, and intergenic); the vertical axis is the total number of methylated CpG sites divided by the total length (in basepairs) of the genomic region. (c) Principal component analysis (PCA) based on

methylation levels at 201,448 CpG sites significantly methylated in at least one sample and covered by 4 or more reads in all samples. (d) Width-scaled violin plots showing CpG methylation levels (vertical axis) per transcriptional plasticity quartile; genes that were expressed and had CpG methylation data (n = 12,368) were binned into quartiles based on mean absolute value of log2-fold-change in expression (a quantification of transcriptional plasticity induced during transfer to different hosts); letters above the quartiles indicate significant difference based on pair-wise Wilcoxon tests (all p-values < 2e-16).

Figure S1: The underside of a 3309 (*V. riparia* x *V. rupestris*) leaf 3 weeks after infestation with *D. vitifoliae*. Small black necrotic lesions formed on the leaves where *D. vitifoliae* attempted to feed.

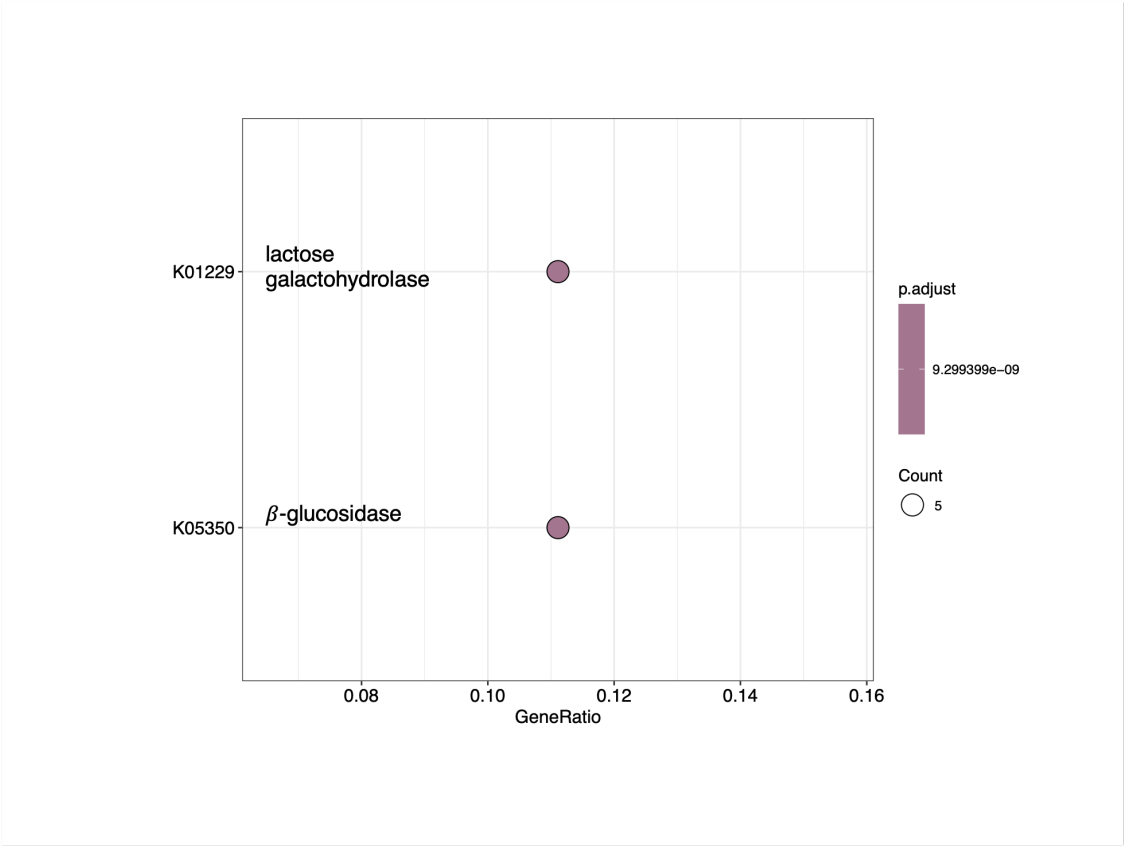

Figure S2: KEGG term enrichment analysis for genes in the darkorange2 co-expression module.

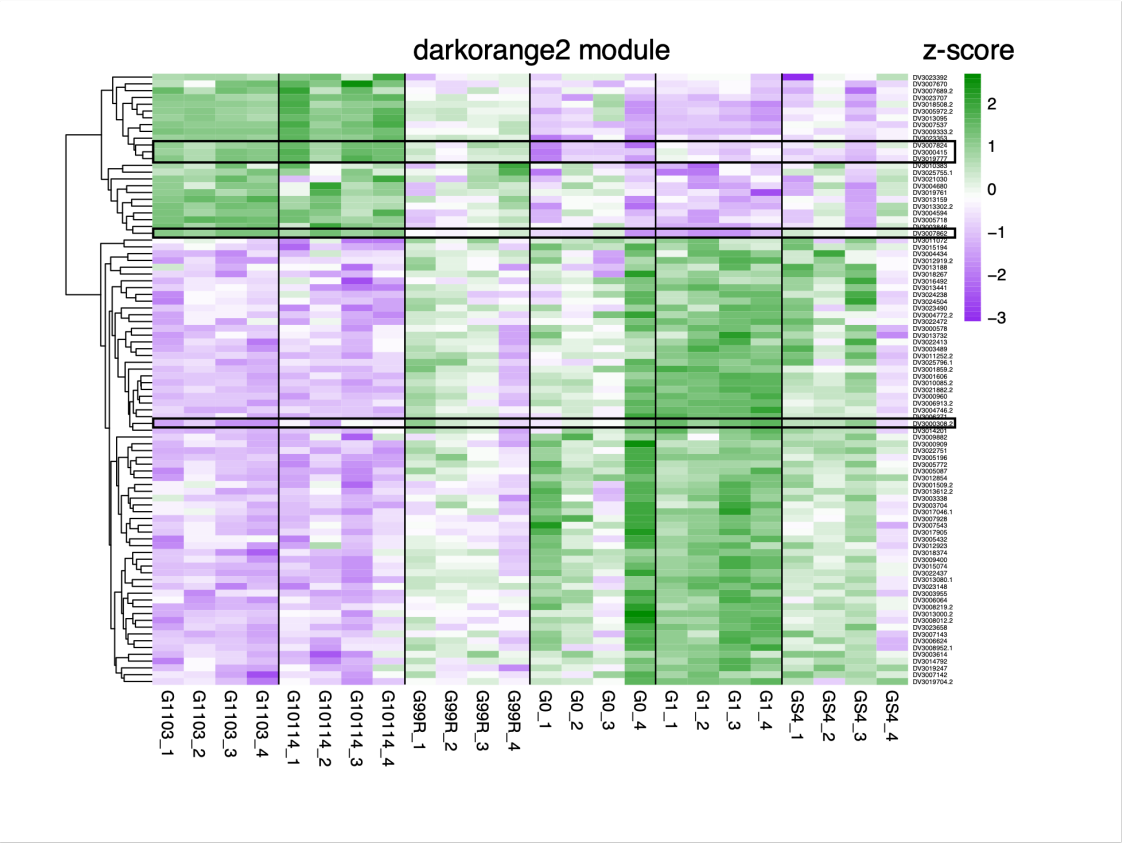

Figure S3: A heatmap of normalized gene expression for genes in the darkorange2 module. Black rectangles highlight genes with glycoside hydrolase KEGG annotation.

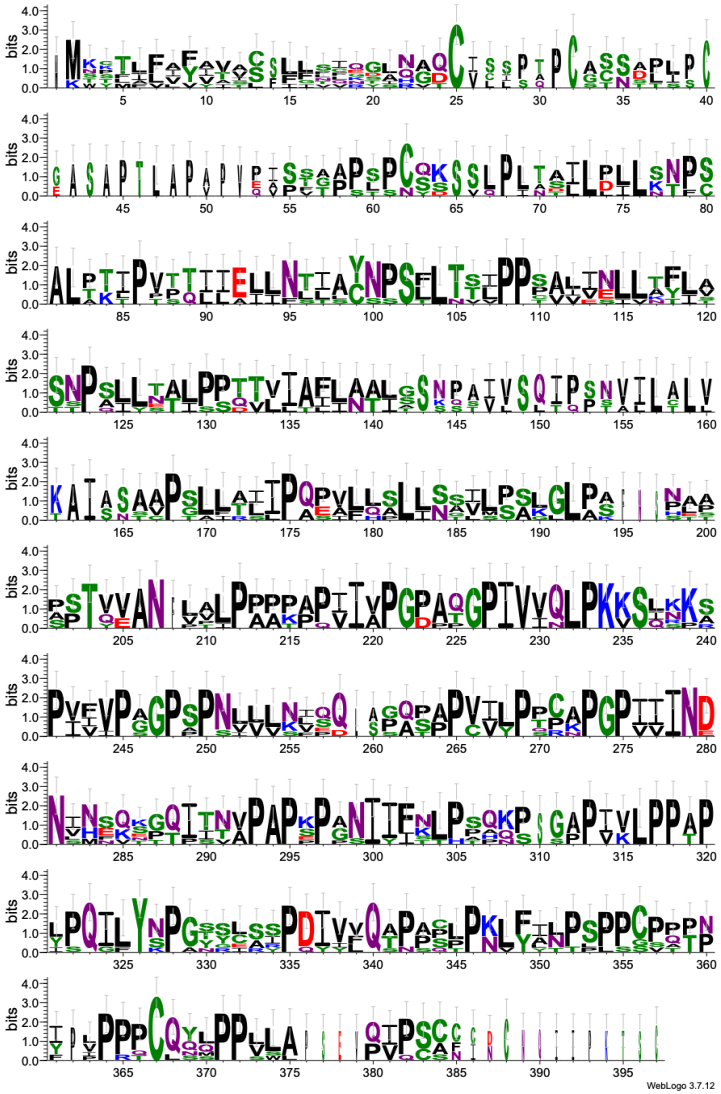

Figure S4: Sequence logo plot based on multiple sequence alignment (MSA) of the seven predicted effector proteins at the ARGL locus.

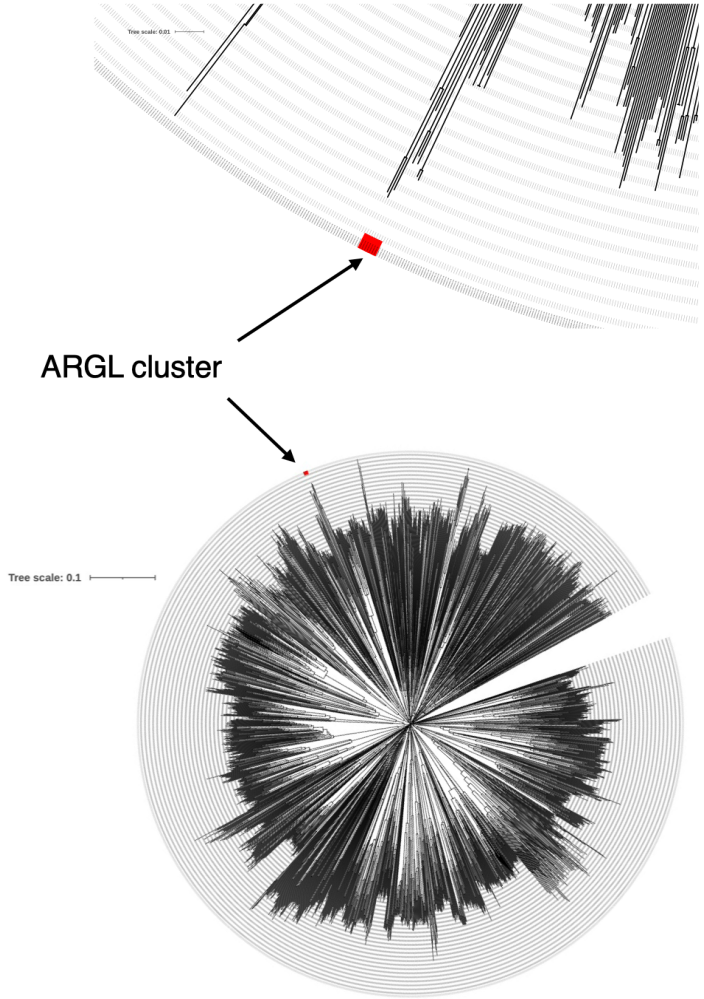

Figure S5: Phylogenetic tree of all 2,743 predicted *D. vitifoliae* effectors based on multiple sequence alignment (MSA) of coding sequences (CDS). ARGL coding sequences are highlighted in red with black arrows.

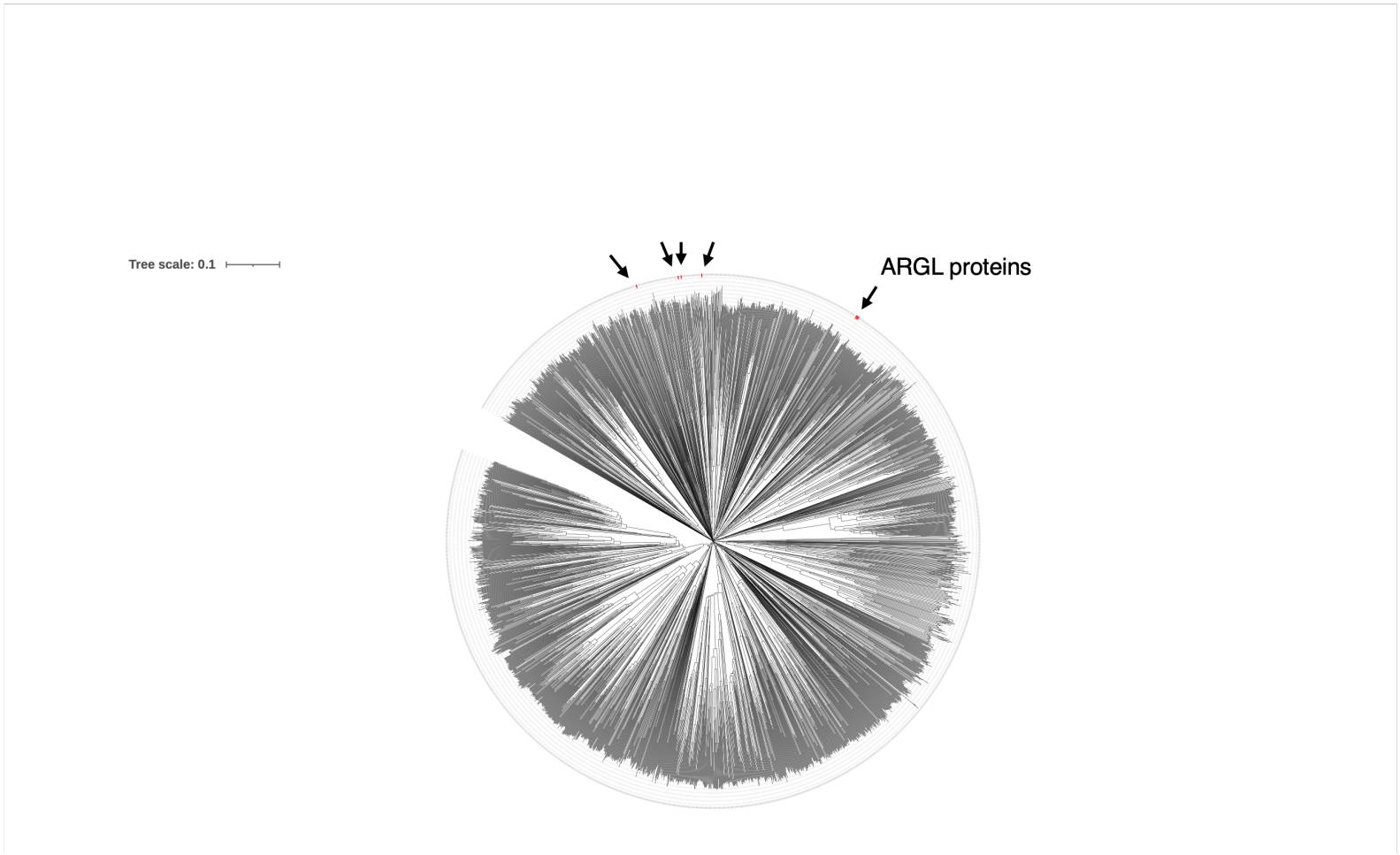

Figure S6: Phylogenetic tree of all 2,743 predicted *D. vitifoliae* effector proteins based on multiple sequence alignment (MSA). ARGL proteins are highlighted in red with black arrows.

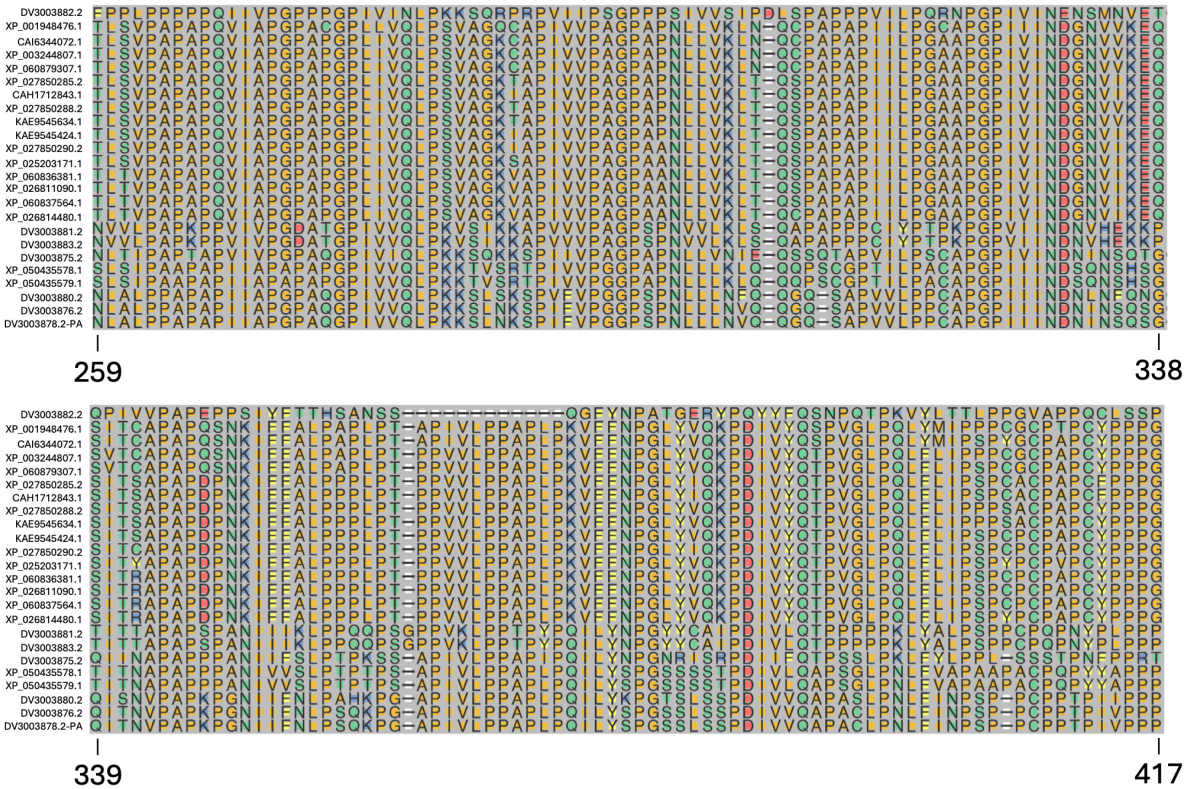

Figure S7: The most conserved region of ARGL proteins. A multiple sequence alignment (MSA) of ARGL proteins was performed using all seven *D. vitifoliae* predicted effector proteins at the ARGL locus and the top seventeen orthologs of ARGL protein DV3003875. Amino acid residues in the 259 to 417 positions are shown, with gene ID (*D. vitifoliae* genes) or accession number on the left.

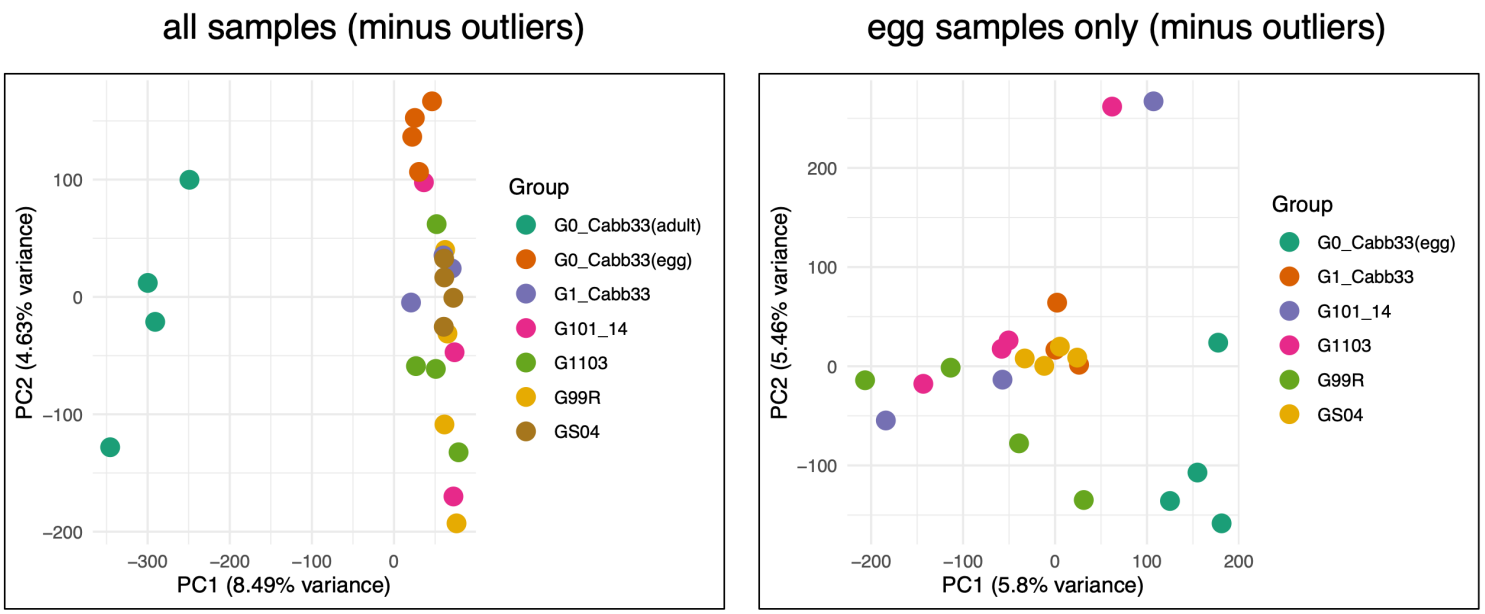

Figure S8: Effect of host genotype on *D. vitifoliae* CpG methylation. Principal component analysis (PCA) based on methylation data for 201,448 CpG sites for all samples minus outliers (left) and only egg samples minus outliers (right).

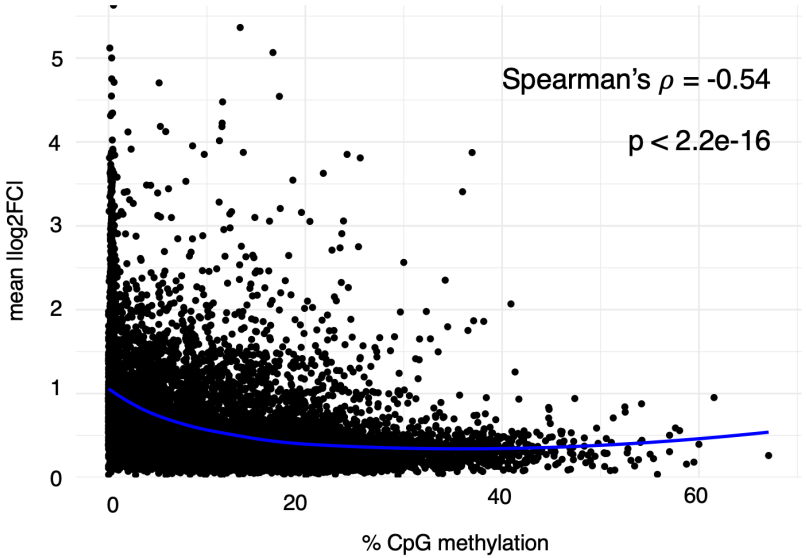

Figure S9: Correlation between transcriptional plasticity and gene body CpG methylation. The scatter plot shows transcriptional plasticity (mean absolute value of log2-fold-change; vertical axis) and percent CpG methylation (horizontal axis) for genes which are expressed and have CpG methylation data (n=12,368). Results of Spearman's rank correlation test are printed in the top right of the plot. A Locally Estimated Scatterplot Smoothing (LOESS) line was fitted to the data (blue line).