Transcriptional variation in glucosinolate biosynthetic genes and inducible responses to aphid herbivory on field-grown *Arabidopsis thaliana*

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Abstract

Recently, increasing attempts have been made to understand how plant genes function *in natura* studies. To determine whether plant defense genes are activated under multiple biotic stimuli, we combined a high-throughput RNA-Seq with insect survey data on 19 accessions of *Arabidopsis thaliana* growing on the field site of Switzerland. We found that genes with GO annotations "glucosinolate biosynthetic process" and "response to insects" were the most significantly enriched, exhibiting largely variable expression among plant accessions. Nearly half of the total expression variation in glucosinolate biosynthetic genes, *AOP*s, *ESM1*, *ESP,* and *TGG1*, was explained by among-accession variance. Combined with the field RNA-Seq data, bioassays confirmed that *AOP3* was up-regulated in response to the mustard aphid *Lipaphis erysimi*. In addition, we also found that the expression of a major cis-jasmone activated gene *CYP81D11* was positively correlated with the number of the flea beetles *Phyllotreta* spp. The combined results from RNA-Seq and insect surveys suggested that plants can activate their defenses even when they are exposed to multiple biotic stimuli *in natura*.

Keywords: *AOP3*; *In natura*; *Lipaphis erysimi*; RNA-Seq; Plant-insect interaction

Abbreviations: ANOVA, analysis of variance; AOP, alkenyl hydroxalkyl producing; ESM1, epithiospecifier modifier 1; ESP, epithiospecifier protein; FDR, false discovery rate; GO, gene ontology; GSL, glucosinolate; MAM, methylthioalkylmalate synthase; rpm, read per million; TGG, thioglucoside glucohydrolase

Footnotes: Sequence data of the present RNA-Seq have been submitted to the NCBI Sequence Read Archive repository, with the accession number, SRP159040.

Introduction

As sessile organisms, plants are exposed to multiple stresses under naturally fluctuating environments (Wilczek et al. 2009; Carrera et al. 2017; Mishra et al. 2017). Recently, growing efforts have been made to understand how plants cope with complex field conditions (Kerwin et al. 2015; Taylor et al. 2017; Carrera et al. 2017; Kono et al. 2017; Sugiyama et al. 2017; Hiraki et al. 2018) and such *in natura* studies are important for the comprehensive understanding of gene functions from the laboratory to the field (Shimizu et al. 2011; Kudoh 2016; Yamasaki et al. 2018; Zaidem et al. 2018; Nagano et al. 2019). Insect herbivores are one of the most diverse organisms that impose biotic stresses on plants (Schoonhoven et al. 2005; Ahuja 2010; Escobar-Bravo et al. 2018). To deal with various threats, plants can activate defense mechanisms only when necessary. Such inducible defenses are triggered by wounding and insect attacks through jasmonate (JA) signaling (Mewis et al. 2005; Escobar-Bravo et al. 2017; Tsuda 2017; Zhu et al. 2018; Zhou et al. 2018; Nakano et al. 2018), while constitutive defenses are continuously expressed.

In the glucosinolate (GSL)-myrosinase system of *Arabidopsis thaliana* and related Brassicales, methionine-derived or aliphatic GSLs confer plant defenses against herbivory (Kliebenstein et al. 2002; Kerwin et al. 2015; Brachi et al. 2015) and possess natural variations in their accumulation and profiles among *A. thaliana* accessions worldwide (Kroymann et al. 2003; Chan et al. 2010; Brachi et al. 2015). The production of aliphatic GSLs is initiated by *MYB28* and *MYB29* transcription factors (Hirai et al. 2007), in which their double mutants accumulate few aliphatic GSLs (Sønderby et al. 2007). During the accumulation of aliphatic GSLs, amino acids and side chain structures are modified by methylthioalkylmalate synthase (MAM), 2-oxoglutarate-dependent dioxygenase encoded in alkenyl hydroxalkyl producing (AOP) loci, and flavin-monooxygenase glucosinolate S-

oxygenase (Kliebenstein et al. 2001; Kroymann et al. 2003; Hansen et al. 2007). The enzyme myrosinase (thioglucoside glucohydrolase, TGG) catalyzes GSL and results in the emission of isothiocyanates, nitriles, or other hydrolysis products when insect herbivores bite plant tissues (Lambrix et al. 2001; Barth and Jander 2006; Zhang et al. 2006; Shirakawa and Hara-Nishimura 2018). Epithiospecifier proteins (ESP also known as TASTY: Lambrix et al. 2001; Jander et al. 2001) promote the hydrolysis of GSL with some modification by the *EPITHIOSPECIFIER MODIFIER1* (*ESM1*) locus (Zhang et al. 2006), resulting in different defense activities against insect herbivores (Ratzka et al. 2002).

Insect herbivores differentially elicit defense responses of a host plant species depending on their feeding habits and host specializations. Leaf chewing herbivores crush plant tissues and accordingly, activate the GSL-myrosinase system (Barth and Jander 2006; Shirakawa and Hara-Nishimura 2018; Martinoia et al. 2018). The hydrolysis products of GSLs act as a toxin against generalist chewers (Lambrix et al. 2001; Kliebenstein et al. 2002; Barth and Jander 2006), whereas specialist herbivores exploit GSL and its hydrolysis products as a host plant signal (Ratzka et al. 2002; Renwick et al. 2006). Sapsuckers, such as aphids and thrips, consume plant fluids and, very rarely, crushes plant tissues (Mewis et al. 2005; Kempema et al. 2007). In addition, damaged plants may emit volatile chemicals that elicit defenses of other individual plants or alter feeding behaviors of other insect species (Bruce et al. 2008; Matthes et al. 2011; Yazaki et al. 2017). To date, *A. thaliana* is known to possess natural variations in secondary metabolisms and inducible defenses (Chan et al. 2010; Snoeren et al. 2010; Routaboul et al. 2012); however, how they function in nature remains poorly understood.

In wild populations, *A. thaliana* has multiple life-cycles within a calendar year (Thompson 1994; Wilczek et al. 2009; Taylor et al. 2017) and is attacked by various

herbivores (Arany et al. 2008; Harvey et al. 2008; Sato et al. 2018). Sato et al. (2018) observed both flowering and vegetative *A. thaliana* co-occurring and being wounded by insect herbivores in a wild population near Zurich, Switzerland in summer. By simulating the summer cohort, our previous study found that 12 insect species, including mustard aphids *Lipaphis erysimi* (Homoptera), flea beetles *Phyllotreta striolata* and *P. atra*. (Coleoptera), diamondback moths *Plutella xylostella* (Lepidoptera), and the western flower thrips *Frankliniella occidentalis* (Thysanoptera), colonized *A. thaliana* and such an insect community composition significantly varied among *A. thaliana* accessions (Sato et al. 2018). We also found that plant trichomes play a key role in altering the abundance of leaf chewing herbivores, but there were unclear correlations between insect abundance and laboratory-measured GSL profiles (Sato et al. 2018). To link insect abundance and plant physiological status in the field, we employed transcriptomics, which were widely used to reveal comprehensive pictures of gene expression (Sun et al. 2017; Xu et al. 2018; Wang et al. 2018; Lin et al. 2018; Want et al. 2018). Here we adopted our previously established protocol of cost-effective RNA-Seq (Nagano et al. 2015; Kamitani et al. 2016; Ishikawa et al. 2017) to the field-grown *A. thaliana*.

The purpose of this study was to reveal to what extent variations in gene expression could be explained by plant genotypes under field conditions, and then to specify which herbivores could modulate plant defense responses. To address these issues, we combined a high-throughput RNA-Seq with insect monitoring data on 19 accessions of *A. thaliana* individuals (Table 1; Figure 1). Such a joint approach using the field transcriptome analysis and insect surveys will provide an overall picture of how *A. thaliana* responds to multiple attackers under natural field conditions.

Results

Insect herbivores on field-grown A. thaliana

We exposed a summer cohort of *A. thaliana* to the field environment in Zurich, Switzerland (47°23'N, 8°33'E) (Figure 1a). As major insect herbivores, we observed *L. erysimi*, *P. striolata*, *P. atra*., *P. xylostella*, and *F. occidentalis*. Of these herbivores *L. erysimi* and *F. occidentalis* are sucking insects, while *P. xylostella* and *Phyllotreta* are leaf chewers (Ahuja 2010; Escobar-Bravo et al. 2018). *Lipaphis erysimi*, *P. xylostella*, and *Phyllotreta* are specialists of Brassicaceae (Ahuja 2010), while *F. occidentalis* is a generalist, which feeds on various plant families (Escobar-Bravo et al. 2018) (Figure 1b).

Gene expression variation among A. thaliana *accessions*

Leaf samples for the RNA-Seq analysis were collected from 183 individual plants. Given that previous laboratory experiments detected inducible defenses 24-48 h after insect attacks (e.g., Mewis et al. 2005; Kuśnierczyk et al. 2008; Matthes et al. 2011), the leaf sampling for RNA-Seq was done 1 d after the final insect monitoring (Figure 1a). We sequenced 92 samples per lane using Illumina HiSeq® 2500 and obtained 829,681 mapped reads per sample on average. After discarding the lower 5% shallow-read samples and excluding genes whose expression level was zero in mean $\log_2$ (rpm + 1) (Figure 1c), 173 individual plants with 24,539 genes were subject to our statistical analyses.

A type III Analysis-of-variance (ANOVA: Sokal and Rolf 2012) was performed to partition whole expression variation into the effects of the plant accession, initial size of individual plants, the presence of a flowering stem, and unexplained residuals (Figure 1c). This variation partitioning showed that, when ordered by its expression variation explained

by each factor, the top 5% of the genes had more than 20% variation attributable to plant accessions (Figure 2a; Table S1). In these highly variable genes, 22 gene ontologies (GO) were significantly enriched at $P_{FDR} < 0.05$ (Table S2). Gene ontology annotation of "response to insect" and "glucosinolate biosynthetic process" were most and second most significantly enriched, respectively (Table 2).

Several key genes of aliphatic GSL biosynthesis, such as *AOP*s (Kliebenstein et al. 2001), showed over half of the expression variation attributable to plant accession (Figure 2a). *AOP2* and *AOP3* are tandemly located in the genome and encode 2-oxoglutarate-dependent dioxygenases that are involved in the side-chain modification of aliphatic GSLs (Kliebenstein et al. 2001). Genes involved in GSL hydrolysis, such as *TGGs*, *ESM1*, and *ESP1* (Lambrix et al. 2001; Zhang et al. 2006; Barth and Jander 2006), also exhibited such a large variation in expression that these genes had nearly a half variation attributable to the plant accession (Figure 2b, e, f, g). *TGG1* and *TGG2* are functionally redundant and their double mutants are known to be susceptible to generalist caterpillars, but not to aphids and specialist caterpillars (Barth and Jander 2006). *ESM1* and *ESP* were initially screened by QTL mapping utilizing the natural variation between the Col and L*er* accession (Lambrix et al. 2001; Jander et al. 2001; Zhang et al. 2006). Furthermore, the transcription factor gene *MYB29*, which is responsible for the high accumulation of aliphatic GSLs (Hirai et al. 2007) showed 32% variation in its expression among field-grown accessions (Figure 2 h).

Inducible response to leaf chewing and sapsucking herbivores

To find candidate genes possessing inducible responses, we included the effects of the number of each herbivore into the ANOVA (Figure 1c). In the ANOVA, the main effect of the number of herbivores (Figure 1c) was used to determine whether the gene expression level

was correlated with herbivore abundance, while the interaction term of the number of herbivores with plant accessions (A×H, Figure 1c) addressed whether the magnitude of the correlation differed among plant accessions. The ANOVA found 27, 25, 20, and 19 candidate genes were significantly related to inducible responses to the mustard aphids, the flea beetles, the diamondback moths, and the western flower thrip, respectively (Table S3).

The expression of *AOP3* was significantly correlated with the number of mustard aphids, *L. erysimi* ($P_{FDR} < 0.001$: Figure 3a, Table S3), indicating that its induction by the aphids depended on background genomic variation. This explained the additional 7% variation in *AOP3* expression. Similar interactions with aphid herbivory were observed for *MYB113* and *JAX1*. Furthermore, *MYB113* is involved in anthocyanin biosynthesis and induced via JA signaling (Gonzalez et al. 2011), and *JAX1* encodes jacalin-type lectin resistance to potexvirus and exhibits varying resistance among natural accessions (Yamaji et al. 2012).

In the same manner, the number of flea beetles (*Phyllotreta* spp.) was positively correlated with the expression level of a major cis-jasmone activated gene, *CYP81D11* (Matthes et al. 2010, 2011), ($P_{FDR} = 0.027$: Figure 3b, Table S3). However, its expression was not correlated with plant accessions (plant accession × beetles, $P_{FDR} = 0.99$), indicating limited effects of background genomic variation. Additionally, the number of leaf holes made by the flea beetles was only related to the expression of three loci of unknown function, AT2G41590 (plant accession × holes, $P_{FDR} < 10^{-6}$), AT1G34844 (plant accession × holes, $P_{FDR} < 10^{-13}$), and AT2G47570 (plant accession × holes, $P_{FDR} = 0.007$).

In addition, AT5G48770, which encodes disease resistance proteins of the TIR-NBS-LRR class family and has GO annotations of “defense response” was expressed in response to the diamondback moth *P. xylostella* (Table S3).

193 Finally, the presence of the western flower thrips, *F. occidentalis*, resulted in the
194 expression of one locus, AT2G15130, that encodes a plant basic secretory protein family
195 protein and has the GO annotation "defense response" (Table S3).
196
197 ***Laboratory bioassay using the specialist aphids***
198 Based on the statistical interactions between plant accession and the number of aphids, we
199 found a positive correlation between *AOP3* and the number of the mustard aphids, *L. erysimi*,
200 in a plant with the Col genomic background (Figure 3a). To determine the possibility of an
201 inducible response of *AOP3* to aphid herbivory, we released *L. erysimi* on Col-0 under
202 controlled conditions and quantified the expression of *AOP3* with RT-qPCR (Figure 4a, b).
203 The bioassay confirmed that the expression of *AOP3* was up-regulated in aphid-infested
204 plants (Wilcoxon rank sum test, $W = 0$, $n = 16$, $P = 0.0002$: Figure 4c).
205
206
207 **Discussion**
208
209 ***Expression variation in glucosinolate biosynthetic genes***
210 Glucosinolate profiles, leaf damage, and plant fitness significantly varied between the GSL
211 wildtype and mutants as shown by a field study on *A. thaliana* (Kerwin et al. 2015). Despite
212 the fact that insect abundance and other environmental conditions were not manipulated, our
213 study detected the GO enrichment of "GSL biosynthesis" and "response to insects" in genes
214 showing the top 5% variation among *A. thaliana* accessions (Table 2). Notably, nearly half of
215 the expression variation in *AOP*s, *ESM*, and *TGG1*, was explained by plant accessions
216 (Figure 2), showing a comparable magnitude of variation with the heritability reported by a

laboratory eQTL study (Wentzell et al. 2007). A third of the expression variation in the transcription factor gene, *MYB29,* was attributable to plant accessions, even though this gene is known to respond to water stress and other abiotic stimuli (Mewis et al. 2012; Martinez-Ballesta et al. 2015; Zhang et al. 2017). Overall, our genome-wide analysis using RNA-Seq indicated that GSL biosynthesis and its anti-herbivore functions were one of the most genetically variable functions in field-grown *A. thaliana*.

Among the GSL biosynthetic genes, *AOP*s showed a remarkably large expression variation among natural accessions. More specifically, L*er*-1 accession expressed *AOP3* and not *AOP2*, while Cvi expressed *AOP2*, but not *AOP3* (Figure 2c, 2d) as reported by Kliebenstein et al. (2001). In the Col accession, *AOP2* encodes non-functional proteins (Kliebenstein et al. 2001), whereas, *AOP3* is not expressed in leaves without herbivory (Schmid et al. 2005). Strong genome-wide associations between the *AOP* loci and GSL profiles have been repeatedly detected among natural accessions cultivated under laboratory (Chan et al. 2010) or controlled greenhouse (Brachi et al. 2015) conditions. Based on a genome scan, Brachi et al. (2015) also detected an adaptive differentiation in the *AOP* loci within the European *A. thaliana*. In this evolutionary context, this study provided field evidence for a link between genomic and functional variations in *AOP*s.

Genes possessing inducible responses to herbivory

Consistent with field RNA-Seq data, our laboratory bioassay revealed that the Col-0 accession had an inducible response in *AOP3* to the mustard aphid *L. erysimi*. This aphid species as well as the generalist and cabbage aphids, *Myzus persicae* and *Brevicoryne brassicae,* are major natural enemies of *A. thaliana* (Züst et al. 2012). In the ecological context, previous studies reported unclear geographical associations between the three aphid

241 species and *AOP*-related chemotypes in Europe, though the geographical distribution of the
242 aphids was linked to that of *MAM*-related chemotypes (Züst et al. 2012). Our previous study
243 also reported no significant correlations between laboratory-measured profiles of aliphatic
244 GSL and the abundance of *L. erysimi* in the field. Microarray analyses and the results of this
245 study, showed that the aphid species differentially induced *AOP3* as this gene could be up-
246 regulated in Col-0 by *L. erysimi*, down-regulated in L*er* by *B. brassicae* (Kuśnierczyk et al.
247 2008), and not induced in Col by *M. persicae* (Kempema et al. 2007). Given its natural
248 variation and response to different aphid species, the inducible response of *AOP*s might be a
249 clue for understanding why *AOP* loci are not tightly linked to the higher phenotypes, such as
250 aphid resistance, in wild populations.
251 Of the genes with the GO annotation of "response to insects", *CYP81D11* exhibited a
252 significantly positive correlation between its expression and the abundance of flea beetles.
253 Previous studies revealed that *CYP81D11* was up-regulated by multiple biotic stimuli,
254 including insect herbivory and pathogen infection (Matthes et al. 2011) via cis-jasmone, a
255 plant volatile emitted by wounding (Bruce et al. 2008; Matthes et al. 2010; Matthes et al.
256 2011). While Matthes et al. (2011) used a Col background as a standard accession, this study
257 included multiple natural accessions and found a positive correlation between flea beetle
258 abundance and *CYP81D11* expression (Figure 3b). However, there was no significant
259 correlation between *CYP81D11* expression and the number of leaf holes made by these
260 beetles. This result was probably because the leaf holes remained on the leaves for a few
261 weeks and did not reflect the timing of wounding. The results of *CYP81D11* indicated that
262 not only herbivory, but also the insect abundance data, were needed to detect its inducible
263 response to flea beetles, exemplifying the importance of detailed ecological observations in *in*
264 *natura* studies.

265

266 ***Conclusion***

267 The combination of insect surveys and field transcriptome analyses led us to observe an
268 inducible defense against insect herbivores on *Arabidopsis thaliana*. These results provide
269 field evidence that the molecular machinery of *Arabidopsis* defense can function in noisy
270 environments. While previous field studies on a Brassicaceae crop reported significant
271 transcriptional changes in response to entire herbivore communities (Broekgaarden et al.
272 2010), our large-scale RNA-Seq and insect monitoring dissected such a transcriptional
273 response to each herbivore. Since the insect species studied here are also known as
274 worldwide herbivores feeding on cultivated and wild Brassicaceae (Yano 1994; Ahuja et al.
275 2010; Sato and Kudoh 2017), our findings may provide molecular insights into Brassicaceae-
276 herbivore interactions *in natura*.

277

278

279 **Materials and Methods**

280

281 ***Field experiment***

282 In the field experiment, we used 17 natural accessions and two glabrous mutants (Table 1),
283 which covers phenotypic variation in trichomes (Larkin et al. 1999; Atwell et al. 2010;
284 Bloomer et al. 2012) and glucosinolates (Chan et al. 2010). We initially prepared 10
285 replicates of the 19 accessions (= 190 plants in total) in an environmental chamber, and then
286 transferred them to the outdoor garden of the University of Zurich at Irchel campus (Zurich,
287 Switzerland: 47º23'N, 8º33'E, alt. ca. 500 m) (Figure 1a). Plants were cultivated using mixed
288 soils of agricultural composts (Profi Substrat Classic CL ED73, Einheitserde Co.) and perlites

289 with a compost to perlite ratio of 3:1 litre volume. No additional fertilizers were supplied
290 because the agricultural soils contained fertilizers. Seeds were sown on the soil and stratified
291 under constant dark conditions at an ambient temperature of 4 ℃ for a week. Plants were
292 grown under short-day conditions (8: 16 h light: dark [L:D] at 20 ℃ and a relative humidity
293 of 60%) for a month. The tray positions were rotated every week to minimize the growth bias
294 based on light conditions. Individual plants were moved to a plastic pot (6.0 × 6.0 × 6.0 cm)
295 and acclimated for 3 days in a shaded area outdoors prior to field experiments. The potted
296 plants were randomly placed in a checkered manner between three blocks where 68, 69, and
297 53 plants were assigned within each block. The potted plants were set on water-permeable
298 plastic sheets without being embedded in the ground (Figure 1a). Each block was more than
299 1.0 m apart from the other and the plants were watered every morning and dawn. These
300 experiments were conducted from July 13 to August 3, 2016.
301 Insects on individual plants were visually counted every 2–3 days. We counted these
302 herbivores and the leaf holes made by the flea beetles. The initial plant size (evaluated by the
303 length of the largest leaf at the start of field experiment: mm) and presence of flowering
304 stems (two weeks after the start of experiment: binary variable) were also recorded so we
305 could consider these phenotypes as covariates of statistical analyses. All monitoring was
306 conducted by a single observer during the daytime (08:00–17:00) for 3 weeks after the
307 beginning of the field experiment to minimize variation. Details of insect abundance and
308 diversity are reported in our previous publication (Sato et al. 2018). In case wounding
309 activated plant defense responses via JA signaling (e.g., Mewis et al. 2005; Broekgaarden et
310 al. 2010; Matthes et al. 2010), we did not sample any leaves until the end of the field
311 experiment.
312

313 ***RNA-Seq experiments and data filtering***

314 Leaves were collected from field-grown plants at the end of the experiment (August 4, 2016).
315 The leaf samples were immediately soaked in RNA preservation buffer (5.3 M $(NH_4)_2SO_4$, 20
316 mM EDTA, 25 mM Trisodium citrate dihydrate, pH 5.2) at 4 ℃ overnight and stored at -
317 80 ℃ until RNA extraction. Total RNA was extracted using the Maxwell 16 Lev Plant RNA
318 Kit (Promega) according to the manufacturer's protocol. Selective depletion of rRNAs and
319 highly abundant transcripts were conducted prior to RNA-Seq library preparations as
320 previously described (Nagano et al. 2015). Then, RNA-Seq library preparation was
321 performed as previously described (Ishikawa et al. 2017). Sequencing using Illumina HiSeq®
322 2500 was carried out by Macrogen Co.

323 The fastq files generated by the sequencing were preprocessed using trimmomatic
324 version 0.32 (Bolger et al. 2014). The preprocessed sequences were mapped on the *A.*
325 *thaliana* reference genome (TAIR10 cDNA) using bowtie version 1.1.1 (Langmead et al.
326 2009) and then quantified using RSEM version 1.2.21 (Li and Dewey 2011). The parameter
327 setting of trimmomatic, bowtie, and RSEM were the same as described by Kamitani et al.
328 (2016). According to Kamitani et al. (2016), we calculated the raw read count and read per
329 million (rpm) from the expected read count generated with RSEM. Transposable elements
330 were excluded prior to statistical analyses. We calculated the total raw read counts for each
331 plant sample and discarded shallow-read samples belonging to the lower 5th percentile of the
332 total raw read counts (Figure 1c). Consequently, samples with more than 12,130 reads were
333 subject to statistical analyses. To exclude non-expressed genes, we then averaged $\log_2$ (rpm +
334 1) for each gene between all plant samples and eliminated genes with a zero average of
335 $\log_2$(rpm + 1) (Figure 1c). Overall, we obtained the final dataset on 24,539 genes for 173
336 plants. In this final dataset, 53 out of 173 samples had $< 10^5$ total reads. However, overall

337 trends did not change when we set the threshold at 10^5, although its statistical power
338 decreased due to the sample size limitation.
339
340 ***Statistical analysis***
341 We used a Type III analysis-of-variance (ANOVA: Sokal and Rolf 2012) to screen genes
342 showing a large expression variation among accessions (Figure 1c). We formulated the liner
343 model as: Y ~ Accession ID (factorial) + Flowering stem (0/1) + Initial leaf length (mm),
344 where Y indicates $\log_2$(rpm + 1) of a focal gene. Sum-of-squares (SS) were calculated to
345 partition expression variation attributable to each explanatory variable. The proportion of
346 expression variation explained by the plant accession was evaluated as SS of the plant
347 accession ID divided by the total SS. Genes in the top 5% of expression variation were
348 selected and subject to statistical analysis, as described below. All statistical analyses were
349 performed using R version 3.2.0 (R Core Team 2015).
350 Subsequently, gene ontology (GO) enrichment analysis was applied to genes
351 showing the top 5% values of the proportion of expression variation explained by the plant
352 accession. The GO.db package (Carlson 2017) and TAIR10 gene annotation were used to
353 build the in-house R script of GO enrichment analysis. The statistical significance of the GO
354 term was determined using Fisher's exact probability tests against the entire database. The *p*-
355 values were adjusted by the false discovery rate (FDR: Benjamini and Hochberg 1995) using
356 the p.adjust function of R. When significant GO terms were detected, the GOBPOFFSPRING
357 database in the GO.db package was used to find the most descendant GO within the
358 Biological Process.
359 We then incorporated the effects of herbivores into an ANOVA to determine whether
360 insect herbivory altered gene expression among plant accessions. We formulated the linear

361 model in ANOVA as: Y ~ Accession ID (factorial) + No. of herbivores + Flowering stem
362 (0/1) + Initial leaf length (mm) + (Accession ID × No. of herbivores), where no. of herbivores
363 referred to the number of individuals of a focal herbivore species. The interaction term,
364 Accession ID × No. of herbivores, represented the non-additive, combined effect exerted by
365 the plant accession and the number of herbivores. This ANOVA was repeated for four major
366 herbivores: The number of mustard aphids *Lipaphis erysimi*, *Phyllotreta* beetles, leaf holes
367 made by *Phyllotreta* spp., diamondback moths *Plutella xylostella*, and western flower thrips
368 *Frankliniella occidentalis*. The number of herbivores was log-transformed to improve
369 normality. Given that previous laboratory experiments detected inducible defenses 24-48 h
370 after insect attacks (e.g., Mewis et al. 2005; Kuśnierczyk et al. 2008; Matthes et al. 2011), we
371 used insect abundance data on August 3, 2016 i.e., a day before RNA sampling as an
372 explanatory variable. The *p*-values were calculated using the *F*-test and corrected by FDR.
373 The aov function implemented in R was used to perform the ANOVA, with each factor
374 dropped from the full model.
375
376 ***Laboratory bioassay and RT-qPCR***
377 To determine whether *A. thaliana* possess inducible responses to the mustard aphid, *L.*
378 *erysimi*, we released this aphid species on Col-0 accessions under controlled conditions in the
379 laboratory, and then quantified the expression of *AOP3* gene in intact and infested plants
380 (Figure 4). *Lipaphis erysimi* were collected from *Rorippa indica* growing in the Seta campus
381 of Ryukoku University, Japan (34º58'N, 135º56'E), and maintained on leaves of *Raphanus*
382 *sativus* var. *longipinnatus* before the bioassay. Seeds were sown in plastic pots (6 cm in
383 diameter and height) filled with moist vermiculate. Four seedlings were kept per pot and
384 others were discarded after germination. Seedlings were grown under 16L: 8D conditions at

385 an ambient temperature of 20 ℃ for a month. Liquid fertilizer was diluted 2000-times diluted
386 and supplied during the cultivation (Hyponex, Hyponex Japan, Osaka; N:P:K = 6:10:5). We
387 assigned two pots to the aphid treatment, while the other two pots were the controls.
388 Approximately 80 wingless aphids were released per pot for the aphid treatment, and the pots
389 were separately covered with an unwoven net. Leaf sampling was conducted once a week
390 after the release of aphids. Leaves were soaked in RNA preservation buffer (5.3 M
391 $(NH_4)_2SO_4$, 20 mM EDTA, 25 mM Trisodium citrate dihydrate, pH 5.2) overnight and stored
392 at -80 ℃ until RNA extraction.
393 Total RNA was extracted using a Maxwell 16 Lev Plant RNA Kit (Promega, Tokyo,
394 Japan) and RNA concentration was measured using a Quant-iT RNA Assay Kit Broad Range
395 (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from 300 ng of the total RNA using
396 the reaction solution composed of 10 μl of template RNA, 4.0 μl of 5× SuperScript IV
397 Reverse Transcriptase buffer (Invitrogen, Carlsbad, CA), 0.5 μl of RNasin® Plus RNase
398 inhibitor (Promega, Tokyo, Japan), 2.0 μl of 100 mM DTT (Invitrogen, Carlsbad, CA), 0.4 μl
399 of 25 mM each dNTP (Clontech, Palo Alto, CA), 0.5 μl of SuperScript IV Reverse
400 Transcriptase (Invitrogen, Carlsbad, CA, USA), and 0.6 μl of 100 μM random primer (N)6
401 (TaKaRa, Kusatsu, Japan), with RNase-free water added up to 20 μl. For the reverse
402 transcription step, the mixture was incubated at 25 ℃ for 10 min, followed by 50 min at
403 56 ℃. SuperScript IV was inactivated by heating the mixture at 75 ℃ for 15 min. The cDNA
404 was 10× diluted with RNase-free water and then RT-qPCR was performed using the Roche
405 LightCycler® 480 with 10 μl reaction solution composed of 2 μl of the template, 5 μl of
406 KAPA SYBR Fast RT-qPCR solution (Kapa Biosystems, Inc., Woburn, MA), 0.5 μl of 10 μM
407 forward and reverse primers, and 2 μl of RNase-free water. The forward and reverse primer
408 of the target gene *AOP3* was 5'-TCAGGGGTCGGTTTTGAAGG-3' and 5'-

GTGAAAGGTTTCGGGCACAC-3', respectively. *ACT2* and *EF-1α* were measured for the internal controls (see Czechowski et al. 2005 for primer sequence). The cDNAs were amplified following denaturation, using the 35-cycle programs (10 s at 95 ℃; 20 s at 63 ℃;10 s at 72 ℃ per cycle). Three technical replicates were set for individual plants and primers. The primer of the target gene *AOP3* was designed based on its full length CDS using NCBI Primer-BLAST with a product length parameter of 50-150 bp. We tried six candidate primers and selected the *AOP3* primer above based on the melting curve of laboratory-grown Col-0 and L*er*-1 accessions. Cp values were calculated following the second derivative maximum method and averaged among three technical replicates. The geometric mean of Cp values between *ACT2* and *EF-1α* was used as the internal control. Delta Cp values were calculated for each individual plant between the target and internal control. Wilcoxon rank sum test was used to test differences in the delta Cp values between the intact and aphid-infested plants.

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433 The authors declare that this study was conducted in the absence of any commercial or
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439
440 **References**
441 1. Ahuja, I., Rohloff, J., and Bones, A.M. (2010) Defence mechanisms of Brassicaceae:
442 implications for plant-insect interactions and potential for integrated pest management: a
443 review. *Agronomy for Sustainable Development* 30: 311–348.
444 2. Arany, A.M., de Jong, T.J., and van der Meijden, E. (2005) Herbivory and abiotic factors
445 affect population dynamics of *Arabidopsis thaliana* in a sand dune area. *Plant Biol.* 7:
446 549–556.
447 3. Atwell, S., Huang, Y.S., Vilhjálmsson, B.J., Willems, G., Horton, M., Li, Y. et al. (2010)
448 Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines.
449 *Nature* 465: 627–631.
450 4. Barth, C., and Jander, G. (2006) *Arabidopsis* myrosinases *TGG1* and *TGG2* have
451 redundant function in glucosinolate breakdown and insect defense. *Plant J.* 46: 549–562.
452 5. Benjamini, Y., and Hochberg, Y. (1995) Controlling the false discovery rate: a practical
453 and powerful approach to multiple testing. *J Royal Stat Soc B.* 57: 289–300.
454 6. Bloomer, R.H., Juenger, T.E., and Symonds, V.V. (2012). Natural variation in *GL1* and its
455 effects on trichome density in *Arabidopsis thaliana*. *Mol. Ecol.* 21: 3501–3515.
456 7. Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for

457 Illumina sequence data. *Bioinformatics* 30: 2114–2120.

458 8. Brachi, B., Meyer, C.G., Villoutreix, R., Platt, A., Morton, T.C., Roux, F., and Bergelson,
459 J. (2015) Coselected genes determine adaptive variation in herbivore resistance
460 throughout the native range of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 112:
461 4032–4037.

462 9. Broekgaarden, C., Poelman, E.H., Voorrips, R.E., Dicke, M., and Vosman, B. (2010)
463 Intraspecific variation in herbivore community composition and transcriptional profiles
464 in field-grown *Brassica oleracea* cultivars. *J. Exp. Bot.* 61: 807–819.

465 10. Bruce ,T.J., Matthes, M.C., Chamberlain, K., Woodcock, C.M., Mohib, A., Webster, B.,
466 Smart,.L.E., Birkett, M.A., Pickett, J.A., and Napier, J.A. (2008) cis-Jasmone induces
467 *Arabidopsis* genes that affect the chemical ecology of multitrophic interactions with
468 aphids and their parasitoids. *Proc. Natl. Acad. Sci. U.S.A.* 105: 4553–4558

469 11. Carlson, M. (2017) GO.db: a set of annotation maps describing the entire Gene Ontology.
470 R package version 3.5.0.

471 12. Carrera, D.Á., Oddsson, S., Grossmann, J., Trachsel, C., and Streb, S. (2017)
472 Comparative proteomic analysis of plant acclimation to six different long-term
473 environmental changes. *Plant Cell Physiol.* 59: 510–526.

474 13. Chan, E.K.F., Rowe, H.C., and Kliebenstein, D.J. (2010) Understanding the evolution of
475 defense metabolites in *Arabidopsis thaliana* using genome-wide association mapping.
476 *Genetics* 185: 991–1007.

477 14. Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K., and Scheible, W. (2005) Genome-
478 wide identification and testing of superior reference genes for transcript normalization in
479 *Arabidopsis*. *Plant Physiol.* 139: 5–17.

480 15. Escobar-Bravo, R., Klinkhamer, P.G., and Leiss, K.A. (2017) Induction of jasmonic acid-

associated defenses by thrips alters host suitability for conspecifics and correlates with increased trichome densities in tomato. *Plant Cell Physiol.* 58: 622–634.

16. Escobar-Bravo, R., Ruijgrok, J., Kim, H.K., Grosser, K., Van Dam, N.M., Klinkhamer, P.G. and Leiss, K.A. (2018) Light intensity-mediated induction of trichome-associated allelochemicals increases resistance against thrips in tomato. *Plant Cell Physiol.* 59: 2462–2475.
17. Gonzalez, A., Zhao, M., Leavitt, J.M., and Lloyd, A.M. (2008) Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in *Arabidopsis* seedlings. *Plant J.* 53: 814–827.
18. Harvey, J.A., Witjes, L.M.A., Benkirane, M., Duyts, H., and Wagenaar, R. (2007) Nutritional suitability and ecological relevance of *Arabidopsis thaliana* and *Brassica oleracea* as foodplants for the cabbage butterfly, *Pieris rapae*. *Plant Ecol.* 189: 117–126.
19. Hansen, B.G., Kliebenstein, D.J., and Halkier, B.A. (2007) Identification of a flavin-monooxygenase as the S-oxygenating enzyme in aliphatic glucosinolate biosynthesis in *Arabidopsis*. *Plant J.* 50: 902–910.
20. Hauser, M.-T., Harr, B., and Schlötterer, C. (2001) Trichome distribution in *Arabidopsis thaliana* and its close relative *Arabidopsis lyrata*: molecular analysis of the candidate gene *GLABROUS1*. *Mol. Biol. Evol.* 18: 1754–1763.
21. Hirai, M.Y., Sugiyama, K., Sawada, Y., Tohge, T., Obayashi, T., Suzuki, A. et al. (2007). Omics-based identification of *Arabidopsis* Myb transcription factors regulating aliphatic glucosinolate biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* 104: 6478–6483.
22. Hiraki, H., Uemura, M., and Kawamura, Y. (2018) Calcium signaling-linked CBF/DREB1 gene Expression was induced depending on the temperature fluctuation in the field: views from the natural condition of cold acclimation. *Plant Cell Physiol.*

https://doi.org/10.1093/pcp/pcy210

23. Ishikawa, T., Kashima, M., Nagano, A.J., Ishikawa-Fujiwara, T., Kamei, Y., Todo, T., and Mori, K. (2017) Unfolded protein response transducer IRE1-mediated signaling independent of XBP1 mRNA splicing is not required for growth and development of medaka fish. *eLife* 6: e26845.
24. Jander, G., Cui, J., Nhan, B., Pierce, N.E., and Ausubel, F.M. (2001) The *TASTY* locus on chromosome 1 of *Arabidopsis* affects feeding of the insect herbivore *Trichoplusia ni*. *Plant Physiol.* 126: 890–898.
25. Kamitani, M., Nagano, A.J., Honjo, M.N., and Kudoh, H. (2016) RNA-Seq reveals virus-virus and virus-plant interactions in nature. *FEMS Microbiol. Ecol.* 92: fiw176.
26. Kempema, L.A., Cui, X., Holzer, F.M., and Walling, L.L. (2007) *Arabidopsis* transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs: similarities and distinctions in responses to aphids. *Plant Physiol.* 143: 849–65.
27. Kerwin, R., Feusier, J., Corwin, J., Rubin, M., Lin, C., Muok, A. et al. (2015) Natural genetic variation in *Arabidopsis thaliana* defense metabolism genes modulates field fitness. *eLife* 4: e05604.
28. Kliebenstein, D.J., Lambrix, V.M., Reichelt, M., Gershenzon, J., and Mitchell-Olds, T. (2001) Gene duplication in the diversification of secondary metabolism: tandem 2-oxoglutarate–dependent dioxygenases control glucosinolate biosynthesis in *Arabidopsis*. *Plant Cell* 13: 681–693.
29. Kliebenstein, D., Pedersen, D., Barker, B., and Mitchell-Olds, T. (2002) Comparative analysis of quantitative trait loci controlling glucosinolates, myrosinase and insect resistance in *Arabidopsis thaliana*. *Genetics* 161: 325–332.
30. Kroymann, J., Donnerhacke, S., Schnabelrauch, D., and Mitchell-Olds, T. (2003)

529 Evolutionary dynamics of an *Arabidopsis* insect resistance quantitative trait locus. *Proc.*
530 *Natl. Acad. Sci. U.S.A.* 100: 14587–14592.
531 31. Kono, M., Yamori, W., Suzuki, Y., and Terashima, I. (2017). Photoprotection of PSI by
532 far-red light against the fluctuating light-induced photoinhibition in *Arabidopsis thaliana*
533 and field-grown plants. *Plant Cell Physiol.* 58: 35–45.
534 32. Kudoh, H. (2016) Molecular phenology in plants: *in natura* systems biology for the
535 comprehensive understanding of seasonal responses under natural environments. *New*
536 *Phytol.* 210: 399–412.
537 33. Kuśnierczyk, A., Winge, P., Jrstad, T.S., Troczyska, J., Rossiter, J.T., and Bones, A.M.
538 (2008) Towards global understanding of plant defence against aphids timing and
539 dynamics of early *Arabidopsis* defence responses to cabbage aphid (*Brevicoryne*
540 *brassicae*) attack. *Plant Cell Environ.* 31: 1097–1115.
541 34. Langmead, B., Trapnell, C., Pop, M., and Salzberg, S.L. (2009) Ultrafast and memory-
542 efficient alignment of short DNA sequences to the human genome. *Genome Biol.* 10:
543 R25.
544 35. Lambrix, V., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D.J., and Gershenzon, J.
545 (2001) The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of
546 glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell* 13: 2793–
547 2807.
548 36. Larkin, J.C., Walker, J.D., Bolognesi-Winfield, A.C., Gray, J.C., and Walker, A.R. (1999)
549 Allele-specific interactions between *ttg* and *gl1* during trichome development in
550 *Arabidopsis thaliana*. *Genetics* 151: 1591–1604.
551 37. Li, B., and Dewey, C.N. (2011) RSEM: accurate transcript quantification from RNA-Seq
552 data with or without a reference genome. *BMC Bioinformatics* 12: 323.

553 38. Lin, C.W., Huang, L.Y., Huang, C.L., Wang, Y.C., Lai, P.H., Wang, H.V. et al. (2017)
554 Common stress transcriptome analysis reveals functional and genomic architecture
555 differences between early and delayed response genes. *Plant Cell Physiol*. 58: 546-559.
556 39. Lin, Y., Jiang, L., Chen, Q., Li, Y., Zhang, Y., Luo, Y. et al. (2018) Comparative
557 Transcriptome profiling analysis of red-and white-fleshed strawberry (Fragaria×
558 ananassa) provides new insight into the regulation of anthocyanins pathway. *Plant Cell*
559 *Physiol*. 59: 1844–1859.
560 40. Martínez-Ballesta, M., Moreno-Fernández, D.A., Castejón, D., Ochando, C., Morandini,
561 P.A., and Carvajal, M. (2015) The impact of the absence of aliphatic glucosinolates on
562 water transport under salt stress in *Arabidopsis thaliana*. *Front. Plant Sci*. 6:
563 https://doi.org/10.3389/fpls.2015.00524
564 41. Martinoia, E., Mimura, T., Hara-Nishimura, I., and Shiratake, K. (2018) The multifaceted
565 roles of plant vacuoles. *Plant Cell Physiol.* 59: 1285–1287.
566 42. Matthes, M.C., Bruce, T.J., Ton, J., Verrier, P.J., Pickett, J.A., and Napier, J.A. (2010)
567 The transcriptome of cis-jasmone-induced resistance in *Arabidopsis thaliana* and its role
568 in indirect defence. *Planta* 232: 1163–1180.
569 43. Matthes, M.C, Bruce, T.J, Chamberlain, K., Pickett, J.A, and Napier, J.A. (2011)
570 Emerging roles in plant defense for cis -jasmone-induced cytochrome P450 CYP81D11.
571 *Plant Signal. Behav.* 6: 563–565.
572 44. Mewis, I., Appel H.M., Hom, A., Raina. R., and Schultz, J.C. (2005) Major signaling
573 pathways modulate *Arabidopsis* glucosinolate accumulation and response to both
574 phloem-feeding and chewing insects. *Plant Physiol.* 138: 1149–1162.
575 45. Mewis, I., Khan, M.A.M., Glawischnig, E., Schreiner, M., and Ulrichs, C. (2012) Water
576 stress and aphid feeding differentially influence metabolite composition in *Arabidopsis*

thaliana (L.). *PLoS ONE* 7: e48661.

46. Mishra, N., Sun, L., Zhu, X., Smith, J., Prakash Srivastava, A., Yang, X. et al. (2017) Overexpression of the rice SUMO E3 ligase gene *OsSIZ1* in cotton enhances drought and heat tolerance, and substantially improves fiber yields in the field under reduced irrigation and rainfed conditions. *Plant Cell Physiol.* 58: 735–746.
47. Nagano, A.J., Honjo, M.N., Mihara, M., Sato, M., and Kudoh, H. (2015) Detection of plant viruses in natural environments by using RNA-Seq. In Plant Virology Protocols (pp. 89-98). Humana Press, New York, NY.
48. Nagano, A.J., Kawagoe, T., Sugisaka, J., Honjo, M.N., Iwayama, K., and Kudoh H. (2019) Annual transcriptome dynamics in natural environments reveals plant seasonal adaptation. *Nat. Plants* https://doi.org/10.1038/s41477-018-0338-z
49. Nakano, M., and Mukaihara, T. (2018) Ralstonia solanacearum Type III effector RipAL targets chloroplasts and induces jasmonic acid production to suppress salicylic acid-mediated defense responses in plants. *Plant Cell Physiol*. 59: 2576–2589.
50. R Core Team. (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/
51. Ratzka, A., Vogel, H., Kliebenstein, D.J., Mitchell-Olds, T., and Kroymann, J. (2002) Disarming the mustard oil bomb. *Proc. Natl. Acad. Sci. U.S.A.* 99: 11223–11228.
52. Renwick, J.A.A., Haribal, M., Gouinguené, S., and Städler, E. (2006) Isothiocyanates stimulating oviposition by the diamondback moth, *Plutella xylostella*. *J. Chem. Ecol.* 32: 755–766.
53. Routaboul, J.-M., Dubos, C., Beck, G., Marquis, C., Bidzinski, P., Loudet, O., and Lepiniec, L. (2012) Metabolite profiling and quantitative genetics of natural variation for flavonoids in *Arabidopsis*. *J. Exp. Bot.* 63: 3749–3764.

601 54. Sato, Y., and Kudoh, H. (2017) Fine-scale frequency differentiation along a herbivory
602 gradient in the trichome dimorphism of a wild *Arabidopsis*. *Ecol. Evol.* 7: 2133–2141.
603 55. Sato, Y., Shimizu-Inatsugi, R., Yamazaki, M., Shimizu, K.K., and Nagano, A.J. (2018)
604 Plant trichomes and a single gene *GLABRA1* contribute to insect community composition
605 on field-grown *Arabidopsis thaliana*. *bioRxiv* 320903.
606 56. Schoonhoven, L.M., van Loon, J.J.A., and Dicke, M. (2005) Insect-plant biology. 2nd ed.
607 Oxford, UK: Oxford University Press.
608 57. Schmid, M., Davison, T.S., Henz, S.R., Pape, U.J., Demar, M., Vingron, M., Schölkopf,
609 B., Weigel, D., and Lohmann, J.U. (2005) A gene expression map of *Arabidopsis*
610 *thaliana* development. *Nat. Genet.* 37: 501–506.
611 58. Shimizu, K.K., Kudoh, H., and Kobayashi, M.J. (2011) Plant sexual reproduction during
612 climate change: gene function *in natura* studied by ecological and evolutionary systems
613 biology. *Ann. Bot.* 108: 777–787.
614 59. Shirakawa, M., and Hara-Nishimura, I. (2018) Specialized vacuoles of myrosin cells:
615 chemical defense strategy in Brassicales plants. *Plant Cell Physiol.* 59: 1309–1316.
616 60. Snoeren, T.A.L., Kappers, I.F., Broekgaarden, C., Mumm, R., Dicke, M., and
617 Bouwmeester, H.J. (2010) Natural variation in herbivore-induced volatiles in
618 *Arabidopsis thaliana*. *J. Exp. Bot.* 61: 3041–3056.
619 61. Sokal, R.R., and Rohlf, F.J. (2012) Biometry, 4th edn New York. NY: WH Freeman and
620 Company.
621 62. Sønderby, I.E., Hansen, B.G., Bjarnholt, N., Ticconi, C., Halkier, B.A., and Kliebenstein,
622 D.J. (2007) A systems biology approach identifies a R2R3 MYB gene subfamily with
623 distinct and overlapping functions in regulation of aliphatic glucosinolates. *PLoS ONE* 2:
624 e1322.

625 63. Sugiyama, A., Yamazaki, Y., Hamamoto, S., Takase, H., and Yazaki, K. (2017) Synthesis
626 and secretion of isoflavones by field-grown soybean. *Plant Cell Physiol.* 58: 1594–1600.
627 64. Sun, H., Chen, L., Li, J., Hu, M., Ullah, A., He, X. et al. (2017) The JASMONATE ZIM-
628 Domain gene family mediates JA signaling and stress response in cotton. *Plant Cell*
629 *Physiol.* 58: 2139–2154.
630 65. Taylor, M.A., Cooper, M.D., Sellamuthu, R., Braun, P., Migneault, A., Browning, A.,
631 Perry, E., and Schmitt, J. (2017) Interacting effects of genetic variation for seed
632 dormancy and flowering time on phenology, life history, and fitness of experimental
633 *Arabidopsis thaliana* populations over multiple generations in the field. *New Phytol.* 216:
634 291–302.
635 66. Thompson, L. (1994) The spatiotemporal effects of nitrogen and litter on the population
636 dynamics of *Arabidopsis thaliana*. *J. Ecol.* 82: 63–88.
637 67. Tsuda, K. (2017) Division of tasks: defense by the spatial separation of antagonistic
638 hormone activities. *Plant Cell Physiol.* 59: 3–4.
639 68. Wang, M., Gu, Z., Wang, R., Guo, J., Ling, N., Firbank, L. G., and Guo, S. (2018) Plant
640 primary metabolism regulated by nitrogen contributes to plant–pathogen interactions.
641 *Plant Cell Physiol*. https://doi.org/10.1093/pcp/pcy211
642 69. Wentzell, A.M., Rowe, H.C., Hansen, B.G., Ticconi, C., Halkier, B.A., and Kliebenstein,
643 D.J. (2007) Linking metabolic QTLs with network and cis-eQTLs controlling
644 biosynthetic pathways. *PLoS Genet.* 3: e162.
645 70. Wilczek, A.M., Roe, J.L., Knapp, M.C., Cooper, M.D., Lopez-Gallego, C., Martin, L.J. et
646 al. (2009) Effects of genetic perturbation on seasonal life history plasticity. *Science* 323:
647 930–934.
648 71. Xu, B., Yu, G., Li, H., Xie, Z., Wen, W., Zhang, J., and Huang, B. (2018) Knockdown of

STAYGREEN in perennial ryegrass (*Lolium perenne* L.) leads to transcriptomic alterations related to suppressed leaf senescence and improved forage quality. *Plant Cell Physiol*. https://doi.org/10.1093/pcp/pcy203

72. Yamaji, Y., Maejima, K., Komatsu, K., Shiraishi, T., Okano, Y., Himeno, M., et al. (2012). Lectin-mediated resistance impairs plant virus infection at the cellular level. *Plant Cell* 24: 778–793.
73. Yamasaki, E., Altermatt, F., Cavender-Bares, J., Schuman, M.C., Zuppinger-Dingley, D., Garonna, I., et al. (2018) Genomics meets remote sensing in global change studies: monitoring and predicting phenology, evolution and biodiversity. *Curr. Opin. Environ. Sust.* 29: 177–186.
74. Yano, S. (1994) Ecological and evolutionary interactions between wild crucifers and their herbivorous insects. *Plant Spec. Biol.* 9: 137–143.
75. Yazaki, K., Arimura, G.I., and Ohnishi, T. (2017) 'Hidden'terpenoids in plants: their biosynthesis, localization and ecological roles. *Plant Cell Physiol.* 58: 1615–1621.
76. Yoshida, Y., Sano, R., Wada, T., Takabayashi, J., and Okada, K. (2009) Jasmonic acid control of *GLABRA3* links inducible defense and trichome patterning in *Arabidopsis*. *Development* 136: 1039–1048.
77. Zaidem, M., Groen, S.C., and Purugganan, M.D. (2018) Evolutionary and ecological functional genomics, from lab to the wild. *Plant J.* https://doi.org/10.1111/tpj.14167
78. Zhang, Z., Ober, J.A., and Kliebenstein, D.J. (2006) The gene controlling the quantitative trait locus *EPITHIOSPECIFIER MODIFIER1* alters glucosinolate hydrolysis and insect resistance in *Arabidopsis*. *Plant Cell* 18: 1524–1536.
79. Zhang, X., Ivanova, A., Vandepoele, K., Radomiljac, J., Van de Velde, J., Berkowitz, O. et al. (2017) The transcription factor *MYB29* is a regulator of *ALTERNATIVE*

673 *OXIDASE1a*. *Plant Physiol.* 173: 1824–1843.

674 80. Zhou, S., Richter, A., and Jander, G. (2018) Beyond defense: multiple functions of

675 benzoxazinoids in maize metabolism. *Plant Cell Physiol.* 59: 1528–1537.

676 81. Zhu, J., Wang, X., Guo, L., Xu, Q., Zhao, S., Li, F., Yan, X., Liu, S., and Wei, C. (2018)

677 Characterization and alternative splicing profiles of the lipoxygenase gene family in tea

678 plant (*Camellia sinensis*). *Plant Cell Physiol.* 59: 1765–1781.

679 82. Züst, T., Heichinger, C., Grossniklaus, U., Harrington, R., Kliebenstein, D.J., and

680 Turnbull, L.A. (2012) Natural enemies drive geographic variation in plant defenses.

681 *Science* 338: 116–119.

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Tables and Figures

Table 1. List of *Arabidopsis thaliana* accessions used in this study.

Accession	ID	Locality	Trichome (no./cm^2)	Aliphatic GSL (nmol/mg)$^{\$}$	
				Short-chain	Long-chain
Bay-0	N22633	Germany	26.3	6.09	2.03
Br-0	N22628	Czech Republic	0	10.69	1.89
C24	N22620	Portugal	2.5	11.1	5.52
Col-0	N22625	USA	32.5	3.1	0.5
Col(*gl1-2*)	CS3126†	USA	4.0‡	NA	NA
Cvi-0	N22614	Cape Verde	104.3	11.18	0.8
Est-1	N22629	Russia	39.3	1.75	0.92
Kas-2	CS6751	India	9	15.5	0.84
Kin-0	N22654	USA	14	13.52	2.22
L*er*-1	N22618	Germany	14.3	7.61	1.16
L*er*(*gl1-1*)	CS64*	Germany	0	NA	NA
Mr-0	N22640	Italy	23.3	14.8	3.2
Ms-0	N22655	Russia	43.6‡	9.83	2.04
Nd-1	N22619	Switzerland	47	9.47	0.59
Se-0	N22646	Spain	30.5	6.62	0.68
Shahdara	N22652	Tajikistan	55.5	9.2	0.83
Tsu-1	N22641	Japan	11.3	14.26	2.18
Van-0	N22627	Canada	20.8	7.85	1.53
Ws-2	N22659	Russia	33.3	7.58	0.76

The table shows stock ID, locality, trichome density (no./cm^2: Atwell et al. 2010), and GSL accumulation (Chan et al. 2010).

*Obtained through the Kiyotaka Okada Laboratory of Kyoto University, Japan.

†Obtained through Dr. M. Ohto.

‡Estimated from the relative trichome density to Col-0 accession presented in previous publications (Hauser et al. 2001 and Yoshida et al. 2009 for Ms-0 and Col(*gl1-2*), respectively)

$^{\$}$Short-chain, the sum of C3- and C4-aliphatic GSLs; Long-chain, the sum of C7- and C8-aliphatic GSLs obtained from Chan et al. (2010).

696 **Table 2.** Gene Ontology (GO) enrichment analysis of genes exhibiting the top 5% expression
697 variation explained by plant accessions. Shown are the top five significant GOs within the
698 biological processes. *P*-values are corrected by the false discovery rate (P_{FDR}). The entire
699 result at P_{FDR} <0.05 is available in Table S2.

GO ID	Term	P_{FDR}
GO:0009625	response to insect	4.76E-05
GO:0019761	glucosinolate biosynthetic process	6.35E-05
GO:0055114	oxidation-reduction process	0.000116
GO:0009627	systemic acquired resistance	0.000327
GO:0009414	response to water deprivation	0.000446

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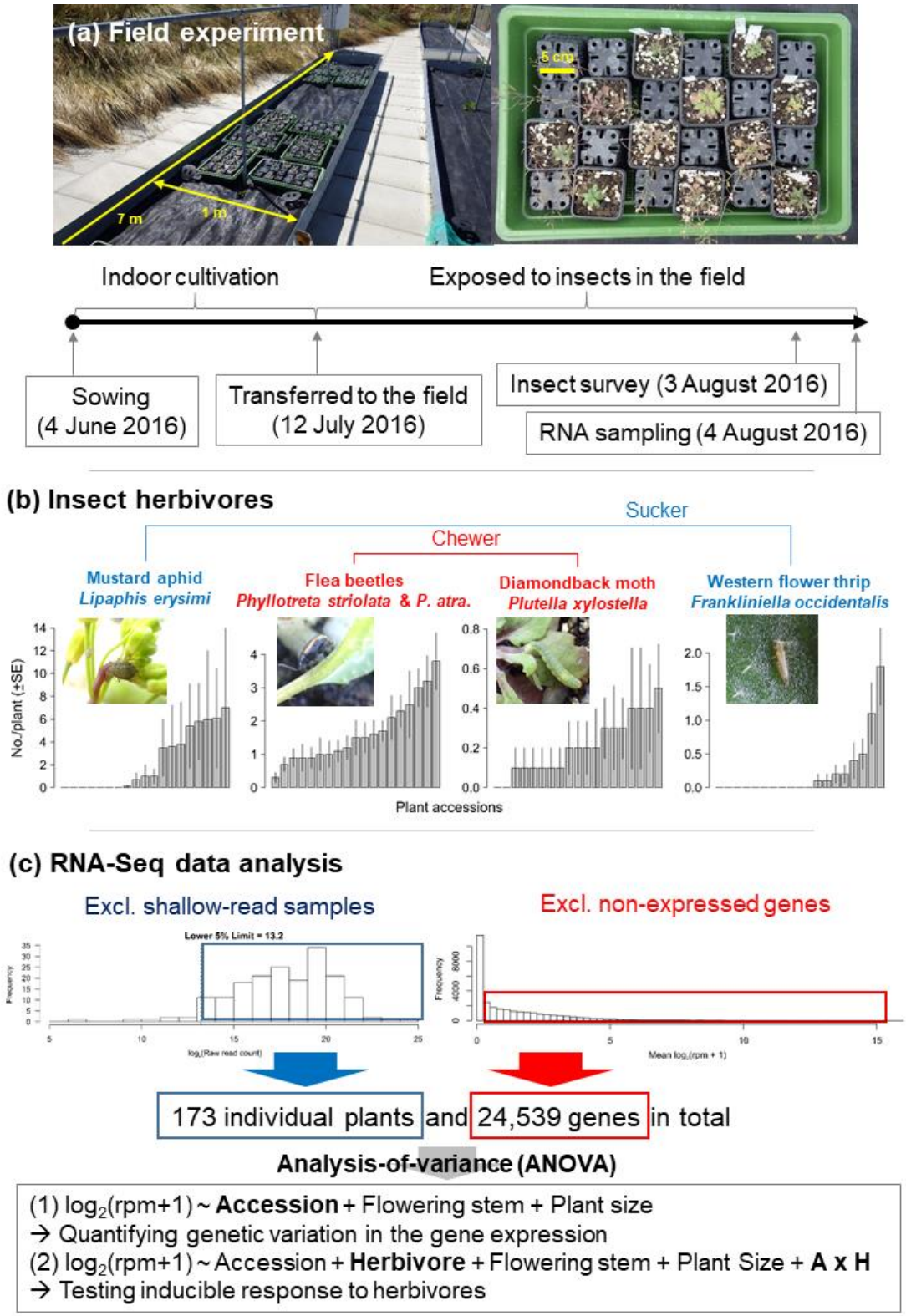

Figure 1. Outline of this field study on *Arabidopsis thaliana*. (a) Procedure of the field experiment. (b) Observed variation in insect abundance among plant accessions. (c) Filtering and statistical analysis of RNA-Seq data. In the formula of ANOVA, the Herbivore represents the main effect of the number of herbivores while the A × H indicates the interaction term between the plant accession and the number of herbivores.

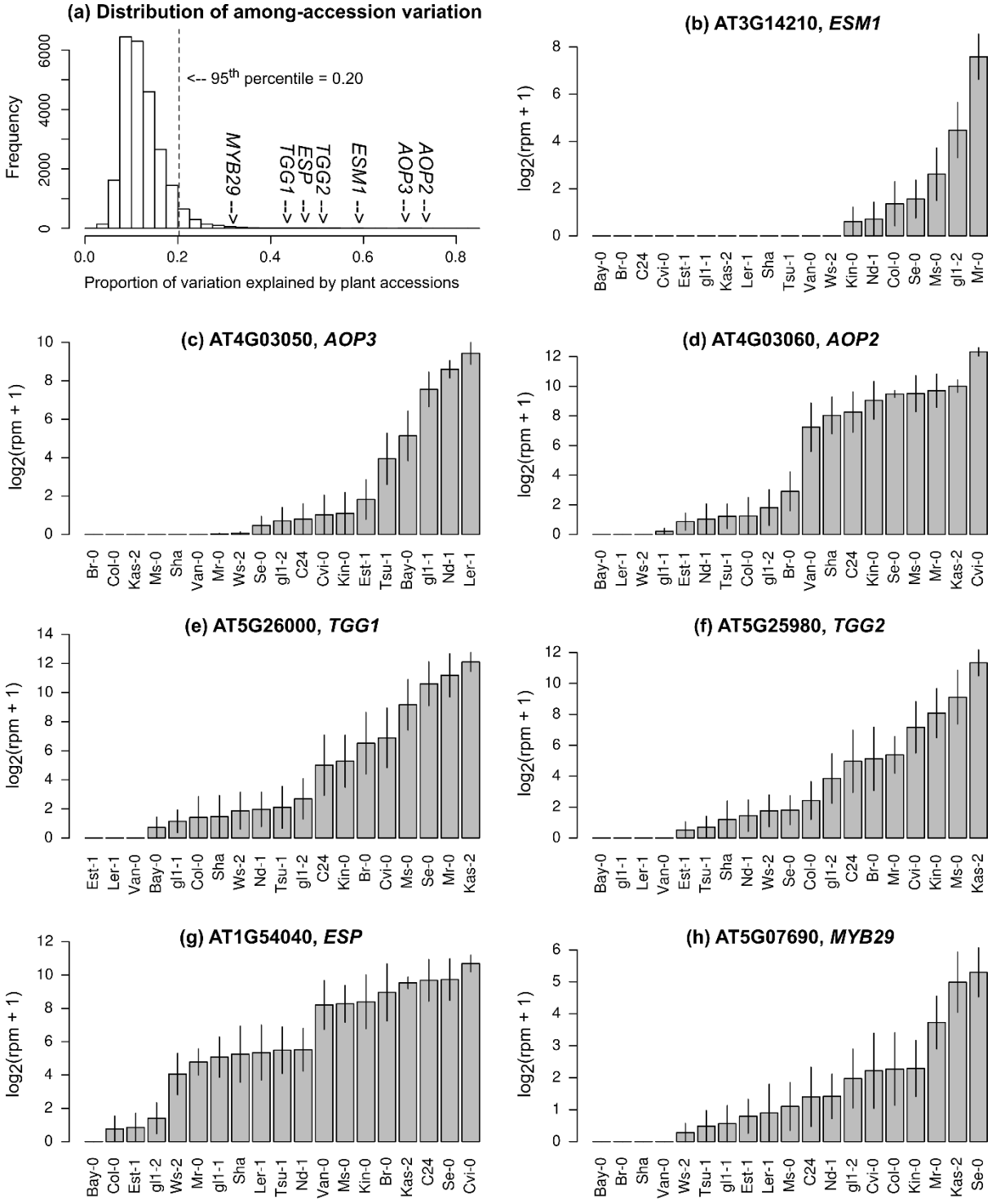

Figure 2. Natural variation in the expression levels of genes involved in glucosinolate biosynthesis and hydrolysis. (a) Histogram showing the proportion of variation explained by plant accessions, (b) the expression of *ESM1*, (c) *AOP3*, (d) *AOP2*, (e) *TGG1*, (f) *TGG2*, (g) *ESP*, and (h) *MYB29*. Grey bars and vertical lines indicate mean ± SE. The list of the top 5% variable genes is available in supporting information (Table S1).

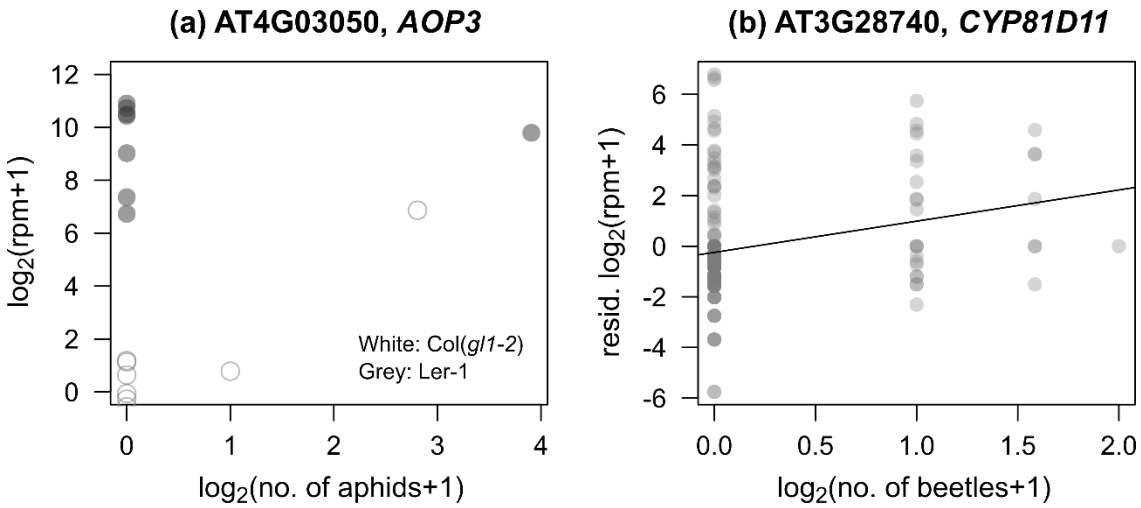

Figure 3. Candidate genes showing a positive relationship between their expressions and insect abundance in the field. (a) Relationship between the number of specialist mustard aphids *Lipaphis erysimi* and the expression of *AOP3* in a constitutively expressed accession L*er*-1 or a potentially induced accession Col(*gl1-2*). (b) Relationship between the number of *Phyllotreta* beetles and the expression of *CYP81D11*. Residuals of $\log_2$(rpm + 1) of *CYP81D11* normalized the expression difference among plant accessions.

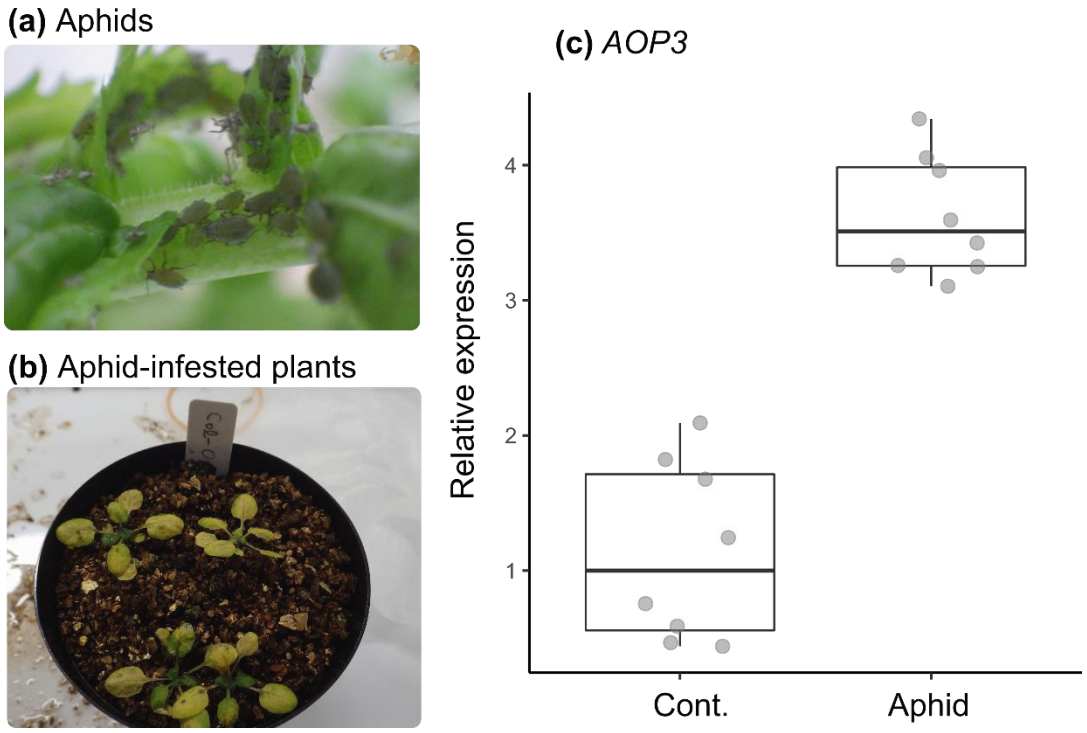

Figure 4. Inducible response of *AOP3* to the specialist mustard aphid *Lipaphis erysimi* in the laboratory-grown Col-0 accession. (a) Photograph of laboratory-reared colony of *L. erysimi*. (b) Aphid-infested seedlings of Col-0 accession. (c) The RT-qPCR analysis of *AOP3* in aphid-infested and control plants. Seedlings are infested by aphids for seven days (Aphid), and no aphids were released for the controls (Cont.).

734 **Supporting information**

735

736 **Table S1.** List of genes showing the top 5% expression variation among *Arabidopsis thaliana*
737 accessions.
738 [TableS1_VarTop5% sheet]

739

740 **Table S2.** Gene ontology (GO) categories enriched at $P_{FDR} < 0.05$ in genes showing the top
741 5% variation explained by plant accessions. *P*-values were corrected by the false discovery
742 rate (P_{FDR}).
743 [Table S2_GO sheet]

744

745 **Table S3.** List of candidate genes possessing inducible response to sapsucking and leaf
746 chewing herbivores at $P_{FDR} < 0.05$. (a) The mustard aphid *Lipaphis erysimi*, (b) the flea
747 beetles *Phyllotreta* spp., (c) the diamondback moth *Plutella xylostella*, and (d) the western
748 flower thrip *Frankliniella occidentalis*. NA means not available. *P*-values were corrected by
749 the false discovery rate (P_{FDR}). Candidate genes are listed for the main effect of each
750 herbivore (Main) and its interactive effect with plant accessions (Interaction).
751 [Table S3_GxE sheet]

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