Plant trichomes and a single gene *GLABRA1* contribute to insect community composition on field-grown *Arabidopsis thaliana*

Yasuhiro Sato[1,2], Rie Shimizu-Inatsugi[3], Misako Yamazaki[3], Kentaro K. Shimizu[3,4*], and Atsushi J. Nagano[5*]

[1]PRESTO, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

[2]Research Institute for Food and Agriculture, Ryukoku University, Yokotani 1-5, Seta Oe-cho, Otsu, Shiga 520-2194, Japan

[3]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

[4]Kihara Institute for Biological Research, Yokohama City University, 641-12 Maioka, 244-0813 Totsuka-ward, Yokohama, Japan

[5]Department of Plant Life Sciences, Faculty of Agriculture, Ryukoku University, Yokotani 1-5, Seta Oe-cho, Otsu, Shiga 520-2194, Japan

[*]Co-corresponding authors: K.K. Shimizu (Phone: +41-44-635-6740) and A.J. Nagano (Phone: +81-77-599-5656)

E-mail address: YS, sato.yasuhiro.36c@kyoto-u.jp; RSI, rie.inatsugi@ieu.uzh.ch; MY, misako.yamazaki@ieu.uzh.ch; KKS, kentaro.shimizu@ieu.uzh.ch; AJN, anagano@agr.ryukoku.ac.jp

Short title: Field study of insects on *Arabidopsis*

Abstract

Background: Genetic variation in plants alters insect abundance and community structure in the field; however, little is known about the importance of a single gene among diverse plant genotypes. In this context, *Arabidopsis* trichomes provide an excellent system to discern the roles of natural variation and a key gene, *GLABRA1*, in shaping insect communities. In this study, we transplanted two independent glabrous mutants (*gl1-1* and *gl1-2*) and 17 natural accessions of *Arabidopsis thaliana* to two localities in Switzerland and Japan.

Results: Fifteen insect species inhabited plant accessions, with 10–30% broad-sense heritability of community indices being detected, such as species richness and diversity. The total abundance of leaf-chewing herbivores was negatively correlated with trichome density at both the field sites, while glucosinolates had variable effects on leaf chewers between the two sites. Interestingly, there was a parallel tendency for the abundance of leaf chewers to be higher on *gl1-1* and *gl1-2* than for their different parental accessions, L*er*-1 and Col-0, respectively. Furthermore, the loss of function in the *GLABRA1* gene significantly decreased the resistance of plants to the two predominant chewers, flea beetles and turnip sawflies.

Conclusions: Overall, our results indicate that insect community composition on *A. thaliana* is heritable across two distant field sites, with *GLABRA1* playing a key role in altering the abundance of leaf-chewing herbivores. Given that such a trichome variation is widely observed in Brassicaceae plants, the present study exemplifies the community-wide impact of a single plant gene on crucifer-feeding insects in the field.

Keywords: Brassicaceae; Community genetics; *GL1*; Herbivory; *In natura*; Plant-insect interaction

Background

Plants develop various resistance traits, such as spines and toxins, to deter herbivory [1]. A growing number of studies on community genetics has revealed that genetic variation in plant resistance traits exerts cascading effects on insect abundance and community composition [2, 3, 4, 5]. These insect indices projected on individual plants, called extended phenotype [5], exhibit heritable variation among plant genotypes [6, 7, 8]. Some researchers have reported the association of particular genetic polymorphisms with leaf damage [9, 10], insect abundance [3, 11], and community composition [3] in the field. In comparison, other studies have focused on how single genes affect the insect community using transformed plants [12, 13]. These lines of evidence from diverse plant species suggest that both quantitative genetic variation and single genes contribute to the community genetics of plant-insect interaction.

Arabidopsis thaliana L. (Brassicaceae) is well-studied as a model system of Brassicaceae-insect interaction [14], within which intensive genomic and phenotypic information is available for a world-wide collection of natural accessions [15]. In *Arabidopsis*-herbivore interactions, plant trichomes (epidermal hairs) function as a mechanical barrier against feeding and oviposition by insect herbivores [11, 16, 17, 18]. Glucosinolates (GLSs) are major secondary metabolites of Brassicales that act as toxic chemicals against generalists [19, 20], but have the potential to attract specialist herbivores [14, 21]. For example, previous studies on *A. thaliana* focused on how these physical and chemical traits confer resistance against specific herbivore species, such as the small cabbage white butterfly *Pieris rapae* [22, 23], the diamond back moth *Plutella xylostella* [16, 20], and the green peach aphid *Myzus persicae* [24, 25]. However, knowledge remains limited about (i) how many insect species occupy *A. thaliana* in the field, (ii) whether plant defense traits contribute to insect abundance and community composition, and (iii) the host genes that are

73 responsible for community members and overall community composition.

74 Laboratory environments are highly constant compared to naturally fluctuating
75 environments; consequently, the phenotype in the laboratory might not be adequate to
76 understand how genes function in the field [26, 27, 28]. The concept of using field studies to
77 determine gene functions (coined *in natura* study: [26, 28]) is applicable to extended
78 phenotypes, such as herbivore abundance and communities on plants. The molecular basis of
79 anti-herbivore defense traits of *A. thaliana* has been studied in laboratory using natural
80 accessions with respect to trichomes [29, 30, 31] and secondary metabolites [20, 32] , and
81 thus provides an ideal opportunity to distinguish the community-wide effects of single genes
82 from naturally existing variation in particular defense traits [33]. For example, trichome
83 density has heritable variation among natural accessions of *A. thaliana* [30, 31, 33]. Loss of
84 function in a transcriptional factor gene, *GLABRA1* (*GL1*, also called *GLABROUS1*) results
85 in glabrous phenotypes on leaf and stem surfaces in *A. thaliana* and related species [34, 35,
86 36, 38, 39] independent of root hair development [34, 40, 41]. Laboratory experiments have
87 shown that the loss of function in *GL1* decreases resistance against leaf-chewing herbivores
88 [22], and improves plant growth by saving the cost of defense [26]. However, these genetic
89 effects remain unexplored in the field.

90 Natural accessions of *A. thaliana* possess various genetic backgrounds regarding
91 their life-cycles in addition to defense traits [31, 42, 43]. In some geographical regions, a
92 rapid life-cycle of *A. thaliana* allows themselves to accomplish two or more generations
93 within a calendar year [42, 43, 44, 45]. The spring cohort of these accessions germinates,
94 flowers, and produces seeds within spring and early summer. The summer cohort
95 subsequently occurs that germinates and spends the summer season at a vegetative stage, and
96 flowers and produce seeds during autumn [43, 44]. These life-cycles of *A. thaliana*

accessions depend on the level of seed dormancy which can be attributed to allelic status in the *DELAY OF GERMINATION1* (*DOG1*) and *DOG6* gene [43, 45, 46], and the duration to flower development which is determined by *FRIGIDA*, *FLOWERING LOCUS C* and several other genes [42, 43, 47]. In wild populations of Europe, *A. thaliana* are attacked by herbivores from late spring to summer: generalist slugs and seed predators occur during late spring, and during summer various insects, such as beetles, moths, aphids, and aphidophagous parasitoids, occupy *A. thaliana* [19, 48, 49]. This seasonal schedule leads us to assume that the summer cohorts of *A. thaliana* should offer an ideal model system between wild *A. thaliana* and diverse herbivorous insects in the field.

Common garden experiments using single-gene mutants provide a powerful tool to determine the causal link between a particular gene and its phenotypes [38, 42, 50, 51]. In this study, we transplanted two glabrous mutants and 17 natural accessions of *A. thaliana*, for which trichome density and glucosinolate concentration vary across plants. In particular, we focused on *gl1-1* and *gl1-2* accessions, of which the former is a null trichome mutant derived from L*er*-1 accession and the latter is a hypomorphic mutant from Col-0 accession [41, 52]. In addition, the natural accessions were selected to cover variation in trichome density, GLSs content, and life-cycles [30, 32]. Common garden experiments with these plants were conducted in the two field sites, Switzerland and Japan, to identify common patterns between the two insect communities. Three specific questions were addressed: (i) is there heritable variation in herbivore abundance and community composition on each *A. thaliana* accession; (ii) which plant trait (physical, chemical, or other life-history traits) does influence herbivore abundance and community composition; (iii) does the loss of function of a single gene *GL1* alter insect abundance and community composition?

Methods

Plant materials and defense traits

Arabidopsis thaliana L., commonly known as thale cress or mouse-ear cress, is an annual weed native to Eurasia and naturalized in North America and East Asia [15]. This species is predominantly self-fertilizing [53] and, when plants are collected from the wild population or when mutants are isolated by mutagenesis, selfed seeds can be maintained as an inbred line called an "accession". Weak dormancy and early-flowering accessions, such as Col-0 and L*er*-1 [45, 46], form both the spring and summer cohort owing to their rapid life-cycle [43]. The spring cohort flowers and seed sets in spring, and then the summer cohort germinates in early summer and flowers in autumn [43]. The accessions with strong dormancy, such as Cvi-0 and Shahdara [46], pass summer as seeds, and furthermore the accession with strong dormancy and late-flowering phenotype, such as Kas-2, are predominantly a winter-annual that has only one generation within a calendar year [43]. In the wild populations, generalist slugs and seed weevils feed on *A. thaliana* during late spring, while more diverse herbivores, such as *Phyllotreta* beetles, green peach aphids *Myzus persicae*, and diamondback moths *Plutella xylostella*, occur during summer [48, 49]. The summer cohort of *A. thaliana* remains at the vegetative stage during summer [43] and thereby provides various herbivores with an opportunity to feed on vegetative plants in the field. We transplanted vegetative *A. thaliana* during June and July to the field sites to simulate the summer cohorts (see 'Common garden experiment' section for details).

To cover wide variation in trichome density (physical defense) and GLS accumulation (chemical defense) with early- and late-flowering cycles, we selected 17 natural accessions and two glabrous mutants (Table 1). The natural accessions selected in this study

should represent the world-wide genetic variation, because the genome-wide pairwise genetic distance was 5.7% in median, which is comparable to that of all accessions analyzed by the 1001 Genome Consortium [15]. These 17 accessions include both early- and late-flowering accessions (e.g. Col-0 and Kas-2 analyzed by Taylor et al. [43]), such that the flowering time under a long-day laboratory condition ranges from 23 (Ws-2 accession) to 92 days (Br-0 accession) [31]. To examine the effects of plant life-history traits on insect community composition, we measured and incorporated the plant size and presence of flowering stem (see 'Common garden experiment' and 'Statistical analysis' below).

To test the functional advantage of *GL1* gene in producing trichomes, we added two glabrous mutants, *gl1-1* and *gl1-2*, to the set of natural accessions (Table 1). The former mutant *gl1-1* has the background of L*er* accession with null mutation due to a 6.5-kb deletion on *GL1* and lacks leaf surface trichomes. The latter *gl1-2* has the background of Col accession with the deletion of 27 amino acid induced by X-ray radiation, showing hypomorphic mutation with lower density of trichome on leaf surface [40, 52]. Out of the 17 natural accessions, Br-0 and C24 have no or few trichomes due to a frameshift mutation and one amino acid change in the myb DNA binding domain of GL1, respectively [30]. We compiled the data of leaf trichome density (no./cm^2) from the GWA-portal (https://gwas.gmi.oeaw.ac.at/: [37]).

All natural accessions were included in previous quantitative genetic studies of GLS, of which seven accessions were used as parental genotypes of recombinant inbred lines (e.g., Col × L*er* and Cvi × L*er* [20]; Bay × Sha [54]; Kas × Tsu [55]) and the other accessions were used in a genome-wide association mapping [34]. To test whether genetic potentials in GLS profiles explain herbivory rate, we used the data in Chan et al. [32] on 21 GLSs of 96 *A. thaliana* accessions using a mature leaf at 35-days post germination from a plant grown under

169 short-day laboratory conditions without herbivory. As they performed two trials to quantify
170 GLS, we used the average GLS contents (nmol/mg flesh weight). We focused on variation in
171 aliphatic GLSs and its chain-length, because these parameters play a major role in preventing
172 above-ground herbivory [50, 56, 57]. Regarding the data of Chan et al. [32], we applied a
173 principal component analysis (PCA) to Total C3-, C4-, C5-, C7-, and C8- aliphatic GLSs. The
174 first and second principal components explained 44% and 33% variation in the GLS profiles
175 among our 17 accessions, respectively (Fig. S1); therefore, these two components were used
176 in our statistical analyses.
177
178 ***Common garden experiment***
179 We used the experimental gardens of the University of Zurich at Irchel campus (Zurich,
180 Switzerland: 47° 23′ N, 8° 33′ E, alt. ca. 500 m) and the Center for Ecological Research,
181 Kyoto University (Otsu, Japan: 35° 06′ N, 134° 56′ E, alt. ca. 200 m) (Fig. 1). The Zurich site
182 is close to a deciduous forest and the surroundings of the common garden are covered with
183 concrete tiles to prevent weeds. The Otsu site is a suburb of cultivated fields and the ground
184 of the study site is covered with short grasses. In the Otsu site, the grass weeds were mown
185 and the surroundings were covered with agricultural sheets before the experiment. At both
186 sites, no large *Brassica* plants occur during early summer. Average air temperature and total
187 precipitation was 19 °C and 198 mm in Zurich (during July 2016; MeteoSwiss,
188 http://www.meteoswiss.admin.ch/home.html) and 22 °C and 321 mm in Otsu (during June
189 2016; Japan Meteorological Agency, http://www.jma.go.jp/jma/index.html).
190 We prepared 10 replicates of 19 accessions (= 190 plants in total) for each
191 experiment. Experimental plants were initially grown in an environmental chamber, and were
192 then transferred to the outside garden. To cultivate plants, we used mixed soils of agricultural

193 composts (Profi Substrat Classic CL ED73, Einheitserde Co. in Switzerland; MetroMix 350,
194 SunGro Co. in Japan) and perlites with a compost to perlite ratio of 3:1 litter volume. No
195 additional fertilizers were supplied because the agricultural soils contain fertilizers. Seeds
196 were sown on the soil and stratified under constant dark conditions at 4–5 °C air temperature
197 for a week. Plants were then grown under short-day condition (8h:16h light:dark [L:D], 20 °C
198 air temperature, and 60% relative humidity) for 1 month to prevent flowering before the field
199 experiment. The plant positions were rotated every week to minimize the growth bias by light
200 condition. Plant individual was moved to a plastic pot (7.5-cm diameter, with 6.5-cm depth in
201 Japan; 6.0 × 6.0 × 6.0 cm in Switzerland), and acclimated for 3 days at shaded outdoor place
202 before the field experiments. The potted plants were randomly placed among three blocks in
203 each common garden: 68, 69, and 53 plants were assigned within each block in Zurich; and
204 76, 76 and 38 plants were assigned within each block in Otsu. The potted plants were set in a
205 checkered manner within a block without being embedded in the ground on water-permeable
206 plastic sheet. Each block was more than 1.0 m apart from each other. These experiments were
207 conducted from June 18 to July 1, 2016 in Otsu; and from July 13 to August 3, 2016 in
208 Zurich. Plants were watered every three days in Otsu and every day in Zurich.
209 Insect and herbivorous collembola on individual plants were visually counted every
210 2–3 days. These species were identified ocularly with a magnifying glass. Dwelling traces
211 and mummified aphids were also counted as a proxy of the number of leaf miners and
212 parasitoid wasps, respectively. Eggs, larvae, and adults were counted for all species, as long
213 as they could be observed by the naked eye. The abundance of each species was evaluated by
214 the cumulative number of individuals over the experimental period to reflect herbivory load
215 on plants [58]. Small holes made by flea beetles were counted at the Zurich site and the
216 maximum number throughout the experiment was used as an indicator of damage by flea

beetles; however, this phenotyping was difficult in Japan, due to heavier and simultaneous infestation by sawflies. We attempted, but failed, to evaluate leaf damage in Japan because about one third of individual plants were dead by the end of the experiment due to high air temperature in June. All counting was conducted by a single observer during the daytime (08:00–17:00), and was continued for 3 weeks after the beginning of the field experiment.

We recorded the initial plant size and presence/absence of flowering stems to incorporate the effects of plant life-history traits on insect abundance. Initial plant size was evaluated by the length of the largest rosette leaf (mm) at the beginning of the field experiment, because this parameter represents plant size at the growth stage. The presence/absence of flowering stems was recorded 2 weeks after transplanting plants.

Statistical analysis

Response variables - Community indices were examined at three levels (i.e., component species, guilds, and entire communities) as response variables in the following analyses. At the species level, we analyzed the number of individuals of each herbivorous species. We analyzed species for which more than 20 individuals were observed in each site, because statistical tests were difficult to apply to rare species. For the Zurich data, we analyzed the number of leaf holes as an indicator of damage by flea beetles. At the guild level, we classified herbivorous species into those feeding on external leaf tissues (i.e., leaf chewers) and those feeding on internal plant tissues (including sap suckers and leaf miners). We also separated herbivorous species into specialists on Brassicaceae (e.g., white butterflies, cabbage sawflies, and turnip flea beetles) and generalists on multiple plant families (some species of aphids and thrips) (Table 2; Fig. S2). The total number of insect individuals in each category was analyzed as guild level statistics. At the entire community level, we calculated species

241 richness (i.e., number of species), Shannon's diversity index *H′*, and the total number of
242 insect individuals on individual plants. All of the response variables were ln(x +
243 1)-transformed to improve a normality before statistical analyses. All statistical analyses were
244 conducted using R version 3.2.0 [59]. We utilized the *rda* function (in the *vegan* package:
245 [60]) to perform the redundancy analysis. We used the *lme* function (in the *nlme* package:
246 [61]) to estimate heritability, as described below.

247 *Variation in insects on plant accessions* - To quantify variation in insect communities
248 among plant accessions and study sites, we performed a redundancy analysis to partition
249 sources of variation in community composition into the plant accession, study sites, and
250 accession-by-site effects. The accession-by-site interaction was first analyzed by 999-times
251 permutation tests, and then the main effects of accessions and sites were examined without
252 the interaction term. Then, we estimated broad-sense heritability H^2 in a focal response, as the
253 proportion of variance attributable to plant accessions. We used liner mixed models, in which
254 the accession ID was assigned as a random effect. This variance component of random effect
255 was estimated by the restricted maximum likelihood method [62, 63]. The significance of
256 heritability was examined by likelihood ratio tests by comparing the linear models with or
257 without the random effect of accession ID. This estimation of heritability was separately
258 performed for the data from Zurich and Otsu. *P*-values were corrected by the false discovery
259 rate (FDR) of multiple testing [64]. Another option of estimating heritability is to incorporate
260 a genetic distance matrix among natural accessions, as used in genome-wide association
261 studies [e.g. 65, 66]. However, it was difficult to apply the same approach to single-gene
262 mutants and the limited number of accessions; thus, we adopted the linear mixed models
263 without the distance matrix to estimate broad-sense heritability.

264 *Effects of plant traits* - To address whether particular plant traits contributed to

community members and composition, we used multiple regressions that considered trichome density, PC1 and PC2 of aliphatic GLSs, the presence/absence of flowering stems, and initial plant size (mm) as explanatory variables. No explanatory variables were heavily correlated with each other ($|r| < 0.6$ for all pairs). We considered the difference of experimental block as a covariate. Because trichome density had a highly skewed distribution, due to completely glabrous phenotypes, this variable was $\ln(x + 1)$-transformed before the analysis. First, we tested the effects of plant traits on each response variable without the dataset on glabrous mutants. When detecting significant effects of trichomes on a particular herbivore, we then compared two glabrous mutants and their parental accessions to test how the *GL1* genes impact guild and community indices encompassing the focal herbivore. Linear mixed models were used to analyze trichome production, initial plant size (mm), and the presence/absence of flowering stems as explanatory variables. The difference in parental background (i.e., L*er*-1 or Col-0) was considered as a random effect. We used the *lme* function with the maximum likelihood method for these mixed models. All of the continuous response and explanatory variables were standardized following a normal distribution, with zero mean and one variance, to make coefficients comparable between the linear models. *P*-values were corrected by FDR [64].

Results

Abundance and communities of insects among plant accessions and study sites

We observed 15 insect species including flea beetles, sawflies, butterflies, moths, aphids, and thrips on *A. thaliana* in the two field experiments (Table 2; Fig. 1). Of these insects, 5 and 3 species were specific to the Otsu and Zurich site, respectively. Redundancy analysis and

permutation tests confirmed that the plant accession, study site, and accession-by-site effects exhibited significant sources of variation in the community composition (Accession, Sum of Squares (SS) = 0.99, $F = 1.57$, $P < 0.001$; Site, SS = 1.31, $F = 36.2$, $P < 0.001$; Accession-by-site, SS = 0.96, $F = 1.54$, $P < 0.001$ with 999 permutations; Fig. 2). We found significant broad-sense heritability in species richness, Shannon diversity, and total abundance of insects on *A. thaliana*, and its magnitude varied between two study sites (10-11% and 15–30% heritability in Zurich and Otsu, respectively: Table 3). When each community member was analyzed separately, we found significant 16% and 33% heritability in the abundance of two predominant herbivores, the striped flea beetle *P. striolata* in Zurich and the turnip sawfly *A. rosae* in Otsu, respectively (Table 3). We detected 40% heritability in the number of leaf holes made by flea beetles in Zurich (Table 3). At both sites, significant heritability was detected in the number of herbivorous individuals for each of the leaf chewer, specialist, and generalist guilds rather than in single species (Table 3). Even when the two mutants *gl1-1* and *gl1-2* were eliminated from our dataset, heritability remained at a similar level with respect to the abundance of the two predominant herbivore species (striped flea beetle in Zurich, $H^2 = 0.20$, LR-$\chi^2_1 = 16.3$, $P_{fdr} < 0.001$; turnip sawfly in Otsu, $H^2 = 0.31$, LR-$\chi^2_1 = 32.3$, $P_{fdr} < 10^{-6}$), the abundance of leaf-chewing herbivores (Zurich, $H^2 = 0.13$, LR-$\chi^2_1 = 14.2$, $P_{fdr} < 0.001$; Otsu, $H^2 = 0.28$, LR-$\chi^2_1 = 27.6$, $P_{fdr} < 10^{-6}$), and insect species richness (Zurich, $H^2 = 0.14$, LR-$\chi^2_1 = 8.7$, $P_{fdr} < 0.01$; Otsu, $H^2 = 0.28$, LR-$\chi^2_1 = 27.3$, $P_{fdr} < 10^{-6}$).

Plant traits underlying the abundance and communities of insects

We examined whether the species, guild, and community structure of insects were affected by trichome density, glucosinolates, and life-history traits among natural accessions (Fig. 3).

313 Significant effects of trichomes at the species and guild levels were observed (Fig. 3; Table
314 S1). Two predominant leaf chewers, the striped flea beetle in Zurich and the turnip sawfly in
315 Otsu, occurred less on hairy accessions than on accessions that produced low quantities of
316 trichomes (Fig. 3, 4b; Table S1). The number of leaf holes was smaller on hairy plants
317 compared to glabrous plants at the Zurich site (Fig. 3, 4a; Table S1), indicating that trichomes
318 have a resistance function against flea beetles. At the Otsu site, the abundance of the eggs and
319 larvae of the small cabbage white butterfly *Pieris rapae* was also low on hairy plants (Fig. 3;
320 Table S1). At the guild level, trichomes had significant negative effects on the leaf chewers at
321 both sites (Fig. 3, 4c, 4d; Table S1). In contrast to trichomes, aliphatic GLSs did not have any
322 consistent effects on herbivore abundance. The first principal component of GLSs had
323 negative effects on leaf chewers, specialist herbivores, species richness, and total abundance
324 at the Zurich site but no significant effects on these indices at the Otsu site (Fig. 3). The
325 second principal component of GLSs was positively correlated with the abundance of turnip
326 sawfly at the Otsu site and western flower thrips at the Zurich site (Fig. 3: Table S1). These
327 effects of trichomes and GLSs were variable between the two sites with respect to insect
328 richness, Shannon diversity, and total abundance (Fig. 3, 4e, 4f; Table S1). Initial plant size or
329 the presence of flowering stems significantly increased insect richness, diversity, and total
330 abundance at the both sites (Fig. 3; Table S1). The result that Kas-2 in Otsu and C24 in
331 Zurich were less likely occupied by leaf chewers (Fig. 4) was due to its small plant size.
332
333 ***Comparing glabrous mutants and parental hairy accessions***
334 We examined the effects of a single gene *GLABRA1* on herbivory, guild, and community
335 indices encompassing two predominant leaf chewers, flea beetles and sawflies. At the species
336 level, compared to parental accessions, two glabrous mutants had significantly more leaf

337 holes made by the flea beetles and larvae of the turnip sawfly (Fig. 4a, 4b; Table S2). At the
338 guild level, leaf chewers tended to occur more often on two glabrous mutants than on each of
339 the parental accessions (Fig. 4c, 4d), although this difference was not statistically significant
340 in Zurich (Table S2). Among community indices, total abundance at the Otsu site was
341 significantly lower on glabrous mutants than on hairy parents (Fig. 4e, 4f) (coef. ± SE = -0.46
342 ± 0.16, Z = -2.86, P < 0.01; Table S2).
343
344
345 **Discussion**
346 *Arabidopsis thaliana* is the best-studied plant in laboratories, and is distributed across the
347 temperate region [15]. Several researchers have studied leaf herbivory [17, 50, 56],
348 herbivorous fauna [19, 48, 49, 67], and plant fitness [17, 48, 50, 56] in *A. thaliana* under field
349 conditions; however, quantitative evidence remains limited in relation to insect community
350 composition on this plant species. In the present study, we found that the community
351 composition of 15 arthropod species was a significantly heritable phenotype for *A. thaliana* in
352 the two distant field sites. Importantly, the loss of function of the *GLABRA1* (*GL1*) gene
353 significantly decreased plant resistance against two predominant chewers, flea beetles and
354 sawflies, at the species level. At the guild level, both *gl1-1* and *gl1-2* mutant plants were more
355 likely to be inhabited by leaf chewers than each of their hairy parents L*er*-1 and Col-0 at both
356 the Zurich and Otsu sites (Fig. 1c, 1d). The parallel pattern in *gl1-1* and *gl1-2* suggests that
357 the single gene *GL1* contributes to community composition. These results demonstrate that
358 variation in a single gene contributes towards shaping insect communities through its impacts
359 on leaf-chewing herbivores.
360

Heritable variation in insect community composition

Despite the large difference in insect community composition, we consistently found significant heritability and effects of trichomes in the two field sites. Our estimated heritability seems moderate (Table 3), but is comparable with the other studies reporting less than 50% heritability in insect abundance and community composition [6, 7, 8]. Using plant genotypes propagated from seeds, Johnson and Agrawal [6] showed that heritability in insect species richness on the perennial *Oenothera biennis* ranged from 10 to 40%, depending on habitat conditions. Our present study also used *A. thaliana* accessions with unique genetic backgrounds, resulting in the moderate heritability of species richness and diversity. We also found that the presence of flowering stems or larger plant size increased species richness and diversity (Fig. 3; Table S1). Plant apparency hypothesis posed the importance of such plant life-history traits in anti-herbivore defense [68], and this hypothesis has been recently supported by a meta-analysis [69], comparative study [70], and genome-wide association mapping [65]. In the context of community genetics, plant life-history traits are a key predictor of insect community composition on perennial herbs [6] and woody plants [2]. Our present study supports the plant apparency hypothesis at the community level of insects on *A. thaliana*.

Consistent with our results in the Zurich site, Harvey et al. [49] found that *A. thaliana* plants sown in summer were heavily attacked by *Phyllotreta* beetles and also harbored by diamondback moths and aphids in the Netherlands. By simulating a summer cohort of *A. thaliana* with various background accessions of flowering time and seed dormancy, our present study showed the importance of plant life-history traits in organizing the summer insect community. In the seasonal context, the parental accessions of two glabrous mutants, Col-0 and L*er*-1, have a weak dormancy allele of *DELAY OF*

385 *GERMINATION* (*DOG1*) [46], which allows *A. thaliana* to germinate during summer [43,
386 45] and thus to serve as food plants for summer herbivores in the field. As we have shown the
387 defense advantage of producing leaf trichomes, the present field experiment could represent
388 how the loss of function in *GL1* imposes summer herbivory on weak dormancy accessions.
389 The seasonality of plant defense would motivate us to further study epistasis or pleiotropy
390 among genes involved in antiherbivore defense and seasonal phenology of *A. thaliana*.
391
392 ***Effects of the single gene GLABRA1 on insect abundance***
393 In reverse genetic analysis, multiple independent mutants with a consistent phenotype are
394 required to prove the function of a particular gene. In addition, using multiple genetic
395 backgrounds of parent can also give a strong proof of a gene function. In a previous study, the
396 roles of single genes related to GLS biosynthesis in modulating herbivory were quantified
397 using mutants derived from a single parental accession, Col-0 [50]. Our common garden
398 experiments illustrate the function of *GL1* gene against herbivory *in natura* using two distinct
399 lines, L*er* (*gl1-1*) and Col (*gl1-2*). To date, several studies have reported associations between
400 *GL1* polymorphism and anti-herbivore functions in field populations of *A. lyrata* [9, 10] and
401 *A. halleri* [11, 18]. Plant trichomes also prevent herbivory by sawflies [71] and flea beetles
402 [72, 73] on *Brassica* cultivars. Together with these results, our present results indicate that
403 plant trichomes and a single gene *GL1* play a key role in physical defense against crucifer
404 feeders.
405 Laboratory experiments on single-gene mutants and natural accessions of *A. thaliana*
406 suggested that plants with high trichome density resisted infestation by aphids [25, 74].
407 Under the two tested field conditions, trichomes had no significant effect on the abundance of
408 aphids, possibly because aphids primarily occurred on flowering stems, on which the

409 trichome density is low. In fact, the presence of flowering stems was positively correlated
410 with the abundance of aphids (Table S1). These results support the limited associations
411 between aphid abundance and *GL1* polymorphism detected in field-grown *A. halleri* [11]. In
412 addition, we could not detect any significant effects of trichomes and *GL1* on the abundance
413 of larval *P. xylostella*, even though trichomes prevent adult moths ovipositing on *A. thaliana*
414 under laboratory conditions [16]. Handley et al. [16] focused on several northern accessions
415 of *A. thaliana*, whereas the current experimental setting covers a wider geographical range of
416 natural accessions. A recent genome-wide association study using 350 natural accessions also
417 found no significant association between *GL1* polymorphism and herbivory by *P. xylostella*
418 [66]. Combined with the previous studies, our present results from field-grown *Arabidopsis*
419 exemplify the importance of testing diverse accessions and environmental conditions.
420 Although several studies on *Nicotiana* plants illustrated the effects of single
421 jasmonate signaling genes on herbivore abundance and communities, silencing jasmonate
422 pathway results in complex pleiotropy on multiple defense traits in leaves and flowers [12, 13,
423 75, 76]. In contrast, *Arabidopsis* trichomes have a simple molecular mechanism that allows
424 *GL1* to be a prime candidate gene for physical defense without pleiotropy. Loss of function
425 mutants in a few transcriptional factor genes (including *GLABRA1* (*GL1*), *GLABRA2*,
426 *GLABRA3*, *TRANSPARENT TESTA GLABRA1*) result in glabrous phenotypes in *A. thaliana*.
427 While the loss of function of the latter three genes results in pleiotropic defects in root hairs,
428 the loss of function of *GL1* does not affect root hairs, due to the subfunctionalization of *GL1*
429 and its homolog *WEREWOLF* [34, 35, 41, 52]. Indeed, many independent null or
430 hypomorphic mutations of *GL1* have been reported in natural accessions of *A. thaliana* [30,
431 37]. Of note, the Br-0 and C24 accessions were the most susceptible accessions to leaf
432 chewers at each site (Fig. 4c, 4d), and have disruptive mutations on *GL1* [30, 37]. Based on

433 genetic regulatory systems and natural variation, the present findings on *GL1* confer an
434 evolutionary implication to its functional advantage in producing trichomes against
435 herbivory.
436
437 ***Varying effects of chemical defense on specialist herbivores***
438 Glucosinolates act as a chemical defense against herbivory [19, 20, 56, 57]; however, some
439 specialist herbivores overcome GLSs [20, 57, 77, 78]. Because the insect communities
440 observed here were mainly composed of specialist herbivores (Table 2), aliphatic GLSs might
441 have had variable effects in our present study. Specifically, the striped flea beetle *P. striolata*
442 efficiently sequesters 4-methylthiobutyl from *A. thaliana*, a short-chain aliphatic GLS [78].
443 The larvae of *A. rosae* sawflies also sequester GLSs [77], whereas adults utilize
444 isothiocyanates, which are breakdown products of aliphatic GLSs, to find host plants [21].
445 The sequestration and host-finding might explain the result that some components of GLSs
446 had negative effects whereas the others had no or positive effects on the abundance of
447 specialist herbivores.
448 The present study utilized GLS data quantified under laboratory conditions with an
449 aim to address whether genetically based variation in GLSs is associated with insect
450 communities. However, these genetic potentials might be insufficient to reflect the effects of
451 GLSs on herbivore abundance in the field, due to phenotypic plasticity and induced response
452 of GLSs to herbivory [23, 24, 50]. For example, the green peach aphid *M. persicae* and the
453 cabbage white butterfly *P. rapae* can modify the expression level of the *MAM1* gene, which
454 involves a chain elongation of aliphatic GLS [23, 24]. In a field study, Kerwin et al. [50]
455 found gene-by-environmental effects on GLS profiles and herbivory on *A. thaliana*, and these
456 effects varied considerably among study years and sites. Multi-year surveys are therefore

457 needed to reveal under what conditions GLS profiles contribute to shaping insect community
458 composition.
459
460 ***Conclusion***
461 Our field investigation showed a genetic basis in the insect community assemblage on *A.*
462 *thaliana*, and the advantage of the functional allele of *GL1* in avoiding leaf chewers. In
463 Brassicaceae plants, evidence is accumulating to suggest that genetic variation within a plant
464 species alters insect community composition and, in turn, exerts selection on plant defense [4,
465 79, 80]. Variation in the trichome density is also observed across Brassicaceae plants [71, 72,
466 73], where *GL1* orthologs affect the trichome density [81]. In the context of community
467 genetics, the present study on *GL1* provides evidence of a key gene affecting the community
468 composition of crucifer-feeding insects. Future study should assess the relative importance of
469 single genes and quantitative genetic variation towards a complete understanding of plant
470 genetic effects on insect community assembly.
471
472 **Abbreviation**
473 *GL1*: *GLABRA1*; GLS: glucosinolate; PCA: principal component analysis; RDA: redundancy
474 analysis; FDR: false discovery rate
475
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487

488 **Availability of data and source codes**

489 The data and R source code are included in the online supplementary material
490 (Additional_file1_Data.xls; Additional_file2_Rscript.txt).

491

492 **Authors' contributions**

493 Y.S., R.S.I., and M.Y. performed the field experiment. Y.S. analyzed the data. Y.S., R.S.I.,
494 K.K.S., and A.J.N. designed the project and wrote the manuscript with input from all
495 co-authors.

496

497 **Ethics approval and consent to participate**

498 Not applicable.

499

500 **Consent for publication**

501 Not applicable.

502

503 **Competing interests**

504 The authors declare that they have no competing interests.

505

506 **References**

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712 **Table 1.** *Arabidopsis thaliana* accessions used in this study.

Accession	ID	Locality	Trichome (no./cm^2)
Bay-0	N22633	Germany	26.3
Br-0	N22628	Czech Republic	0
C24	N22620	Portugal	2.5
Col-0	N22625	USA	32.5
Col(*gl1-2*)	CS3126[†]	USA	4.0[‡]
Cvi-0	N22614	Cape Verde	104.3
Est-1	N22629	Russia	39.3
Kas-2	CS6751	India	9
Kin-0	N22654	USA	14
L*er*-1	N22618	Germany	14.3
L*er*(*gl1-1*)	CS64[*]	Germany	0
Mr-0	N22640	Italy	23.3
Ms-0	N22655	Russia	43.6[‡]
Nd-1	N22619	Switzerland	47
Se-0	N22646	Spain	30.5
Shahdara	N22652	Tajikistan	55.5
Tsu-1	N22641	Japan	11.3
Van-0	N22627	Canada	20.8
Ws-2	N22659	Russia	33.3

713 The table shows the stock ID, locality, and trichome density (no./cm^2: Atwell et al. [31]).

714 [*]Obtained through Kiyotaka Okada Laboratory of Kyoto University, Japan.

715 [†]Obtained through Dr. M. Ohto.

716 [‡]Estimated from the relative trichome density to Col-0 accession presented in previous

717 publications (Hauser et al. [37] and Yoshida et al. [36] for Ms-0 and *gl1-2*, respectively)

718 **Table 2.** Insect species observed on field-grown *Arabidopsis thaliana*.

Common name	Scientific name	Feeding habit	Host range	Abundance †	
				Zurich	Otsu
Cabbage looper	*Trichoplusia ni*	Leaf chewer	Generalist	0	-
Diamond back moth	*Plutella xylostella*	Leaf chewer	Specialist	++	++
Garden springtail*	*Bourletiella hortensis*	Leaf chewer	Generalist	-	+
Piggyback grasshopper	*Atractomorpha lata*	Leaf chewer	Generalist	-	-
Small cabbage white butterfly	*Pieris rapae*	Leaf chewer	Specialist	+	+
Striped flea beetle	*Phyllotreta striolata*	Leaf chewer	Specialist	++	-
Turnip flea beetle	*Phyllotreta atra*	Leaf chewer	Specialist	+++	0
(Leaf holes made by flea beetles)	*Phyllotreta* spp.	Leaf chewer	Specialist	+++	-
Turnip sawfly	*Athalia rosae*	Leaf chewer	Specialist	0	+++
(Dwelling traces)	NA	Internal feeder	Generalist	-	0
Green peach aphid	*Myzus persicae*	Internal feeder	Generalist	+	+
Mustard aphids	*Lipaphis erysimi*	Internal feeder	Specialist	+++	+
Onion thrip	*Thrips tabaci*	Internal feeder	Generalist	+	+
Western flower thrip	*Frankliniella occidentalis*	Internal feeder	Generalist	++	++
(Parasitoid wasp indicated by mummified aphids)	NA	Carnivore		+	+
Seven-spot ladybird	*Coccinella septempunctata*	Carnivore		0	-

719 Detailed abundance in the Zurich and Otsu sites is provided in the supplementary material

720 (Figure S2).

721 *Only this species is a non-insect arthropod.

722 †Abundance level: +++ (abundant), ++, +, - (rare), and 0 (not found).

723 NA indicates not applicable.

724

725

726 **Table 3.** Likelihood ratio tests for estimating broad-sense heritability H^2.

Response	Zurich			Otsu		
	H^2	LR-χ^2	P_{fdr}	H^2	LR-χ^2	P_{fdr}
Turnip sawfly	---	---	---	**0.33**	**41.0**	**2.0.E-09**
Leaf holes	**0.40**	**55.1**	**1.5.E-12**	---	---	---
Striped flea beetle	**0.16**	**12.6**	**1.2.E-03**	---	---	---
Turnip flea beetle	0.01	0.16	0.75	---	---	---
Diamond back moth	1.6E-09	-6.5E-08	1	0.07	2.87	0.13
Small cabbage white butterfly	---	---	---	0.05	1.92	0.22
Green peach aphid	---	---	---	0.03	0.55	0.50
Mustard aphid	0.04	1.30	0.32	0.03	0.48	0.50
Western flower thrip	**0.32**	**38.8**	**3.1.E-09**	0.03	0.46	0.50
Leaf chewer	**0.14**	**10.4**	**0.003**	**0.28**	**31.3**	**9.4.E-08**
Internal feeder	0.04	1.22	0.32	**0.14**	**9.7**	**0.003**
Specialist	**0.11**	**6.32**	**0.02**	**0.25**	**25.8**	**1.0.E-06**
Generalist	**0.17**	**13.2**	**0.001**	**0.12**	**7.5**	**0.01**
Species richness	**0.11**	**6.82**	**0.02**	**0.26**	**27.3**	**5.8.E-07**
Shannon diversity	**0.11**	**6.10**	**0.02**	**0.17**	**13.4**	**0.001**
Total abundance	**0.10**	**5.97**	**0.02**	**0.29**	**33.0**	**5.9.E-08**

727 Likelihood ratio, LR-χ^2, was tested by comparing the models with and without a random
728 effect of the accession ID. *P*-values were based on a χ^2 distribution with one degree of
729 freedom and corrected by false discovery rate, FDR [64]. Bold values indicate significant H^2
730 at $P_{fdr} < 0.05$. Bars indicate no information available due to low abundance.
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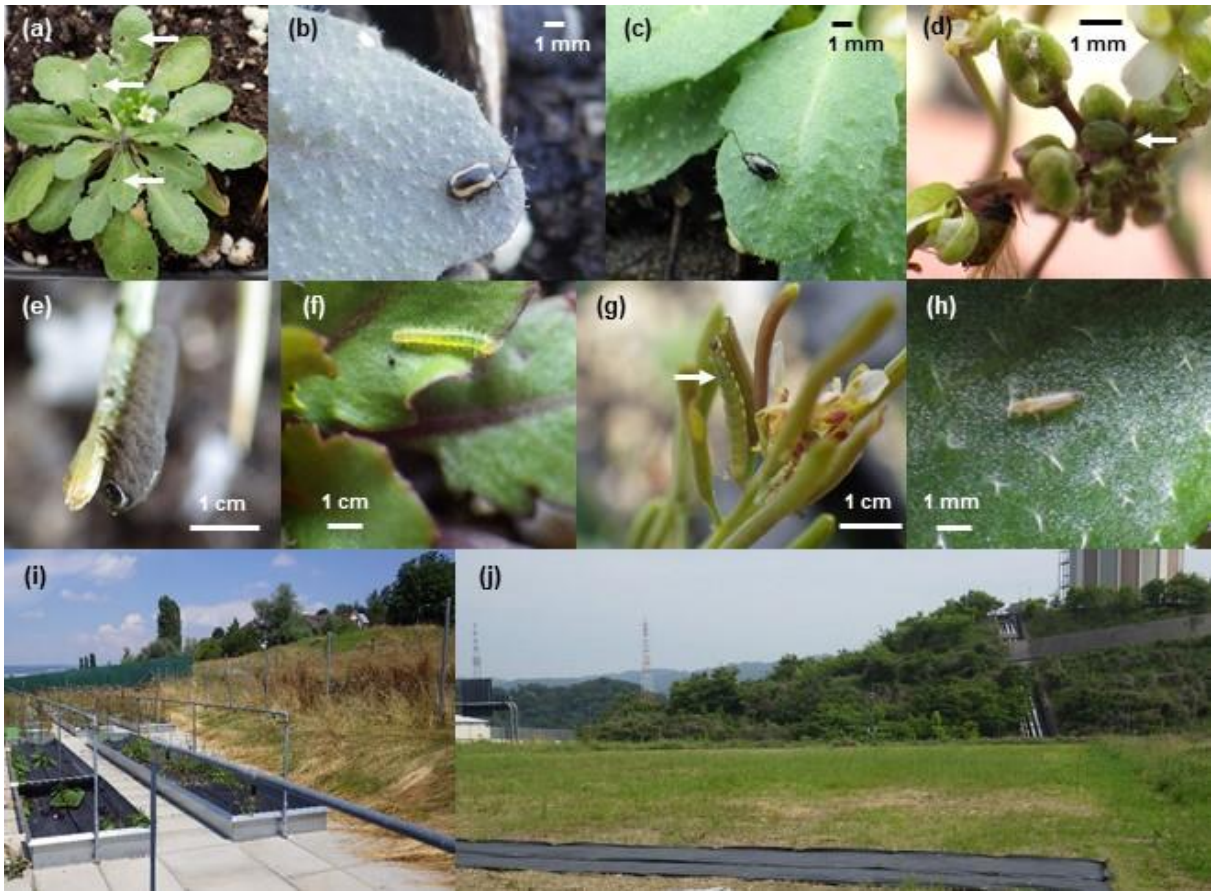

735 **Figure 1.** Photographs of plants and insects. **(a)** Leaf holes made by flea beetles (arrows), **(b)**
736 a striped flea beetle *Phyllotreta striolata*, **(c)** a turnip flea beetle *Phyllotreta atra*, **(d)** mustard
737 aphids *Lipaphis erysimi*, **(e)** a larva of the turnip sawfly *Athalia rosae*, **(f)** a newly hatched
738 larva of the small cabbage white butterfly *Pieris rapae* **(g)** a larva of the diamond back moth
739 *Plutella xylostella*, **(h)** a western flower thrips *Frankliniella occidentalis*, **(i)** the field site in
740 Zurich, Switzerland, and **(j)** the field site in Otsu, Japan.
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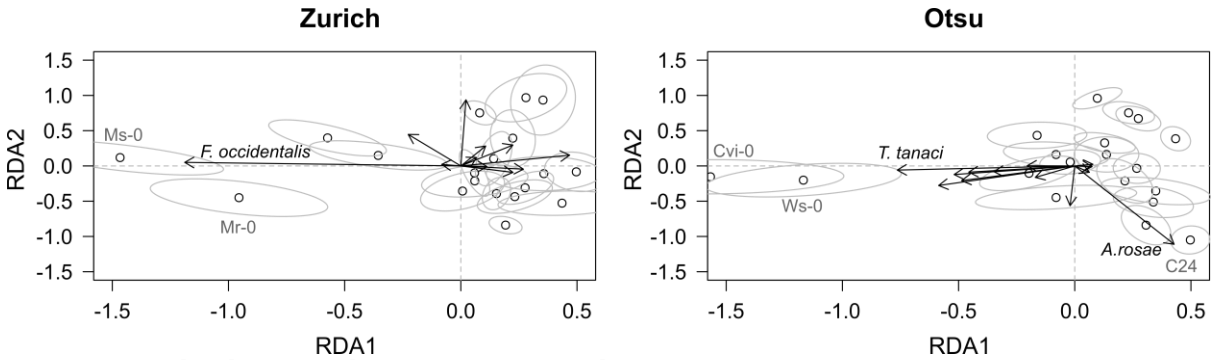


Figure 2. Redundancy analysis summarizing the community composition among 19 accessions of *A. thaliana* in Zurich, Switzerland and Otsu, Japan. White and grey circles indicate the accession mean and its standard error projected on the first and second RDA dimension. Arrows represent the contributions of each species. Permutation tests confirmed significant variation in the community composition among plant accessions and study sites (see the Results section).

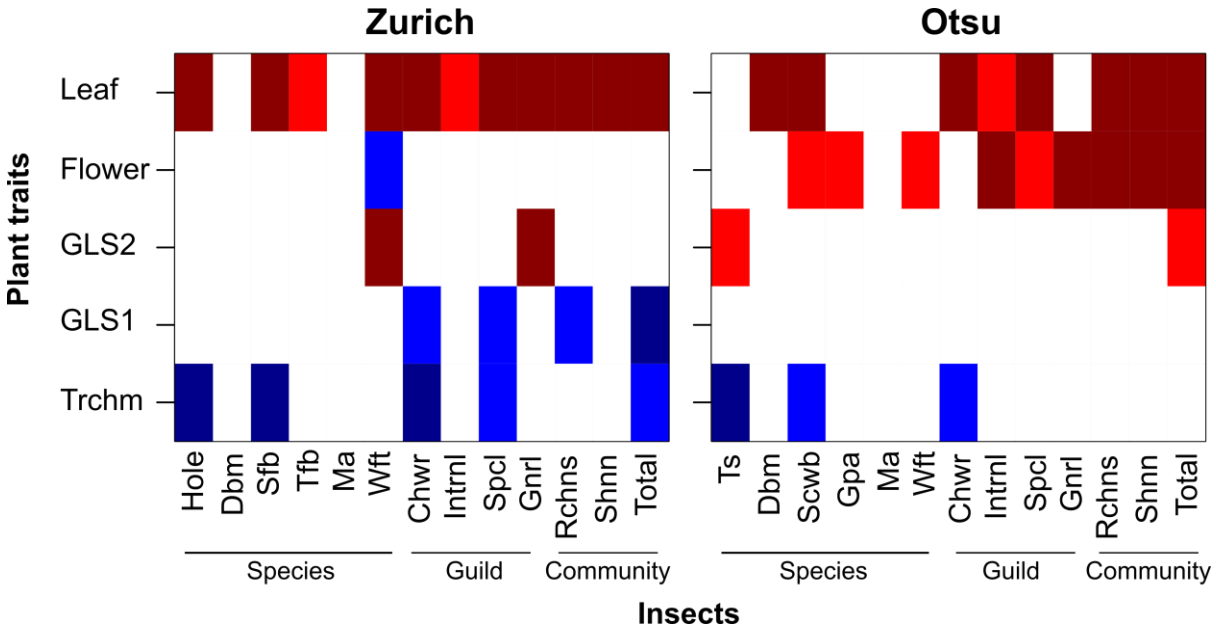
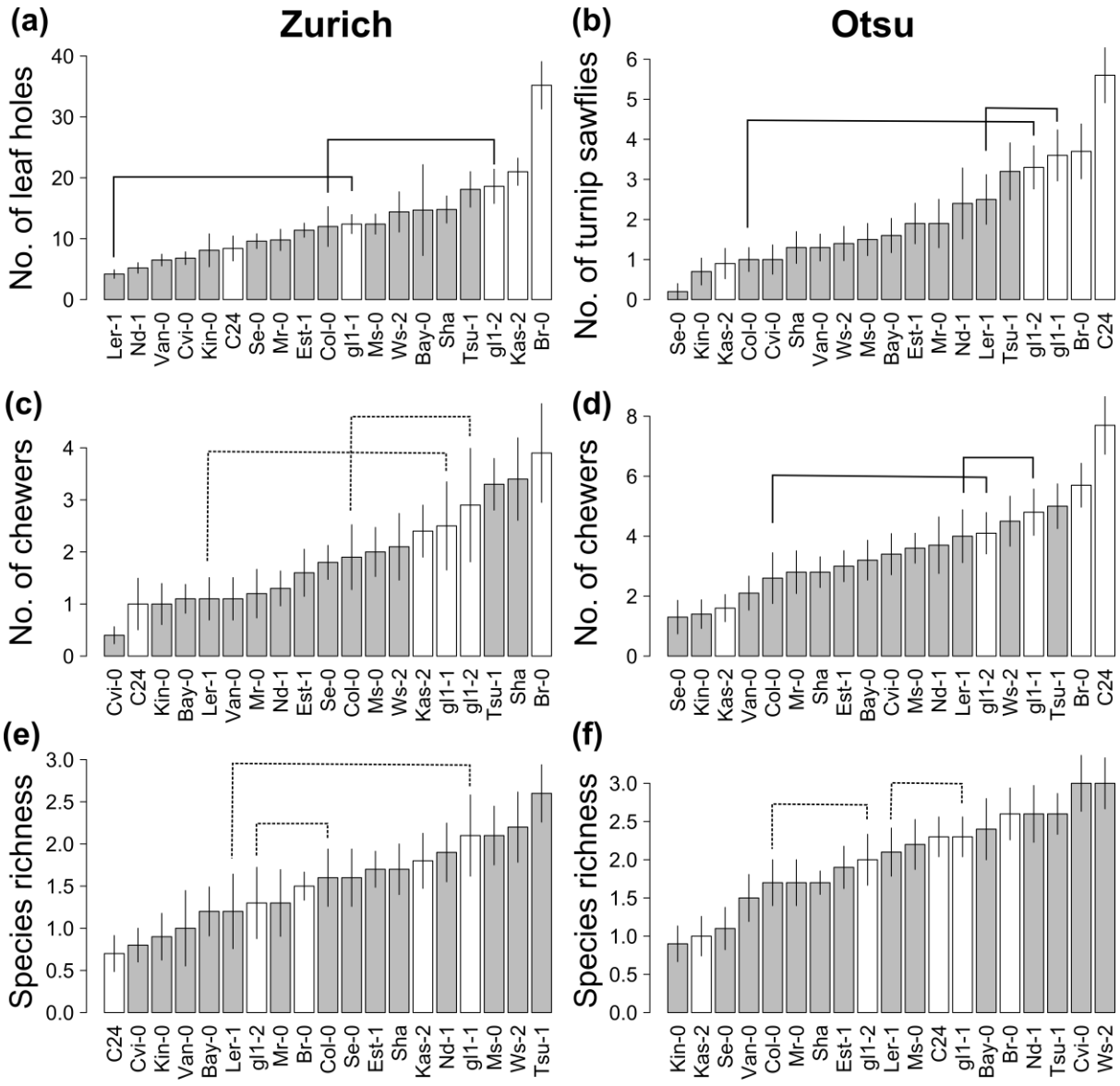


Figure 3. A heat map showing FDR-corrected p-values (P_{fdr}) for the effects of plant traits on insect species, guild, and community indices among 17 natural accessions in Zurich, Switzerland and Otsu, Japan. Shown are the effects of trichome density (Trchm), PC1 and PC2 of aliphatic glucosinolates (GLS1 and GLS2), presence of flowering stems (Flower), and initial leaf length (Leaf) on the diamond back moth (Dbm), striped flea beetle (Sfb), turnip flea beetle (Tfb), mustard aphid (Ma), western flower thrip (Wft), turnip sawfly (Ts), small cabbage white butterfly (Scw), green peach aphid (Gpa), leaf chewers (Chwr), internal feeders (Intrnl), specialists (Spcl), generalists (Gnrl), species richness (Rchns), Shannon diversity (Shnn), and total abundance (Total). Colors represent the sign and significance of trait effects: ■(dark blue), - coef. with $P_{fdr} < 0.01$; ■(blue), - coef. with $P_{fdr} < 0.05$; ■(dark red), + coef. with $P_{fdr} < 0.01$; ■(red), + coef. with $P_{fdr} < 0.05$; □(white), not significant at $P_{fdr} > 0.05$.



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778 **Figure 4.** Variation in insect species, guild, and community on 19 *A. thaliana* accessions
779 (mean ± SE) in Zurich, Switzerland (left panels) and Otsu, Japan (right panels). Given the
780 significant effects of trichomes on flea beetles *P. striolata* and sawflies *A. rosae* (Fig. 3),
781 these panels show herbivory, guild, and community indices comprising the flea beetles and
782 sawflies. White bars of plant accessions represent the sparse density of less than 10 leaf
783 trichomes/cm^2. Connected lines highlight pairs between a glabrous mutant and its parental
784 accession, where solid and dashed lines indicate significant and non-significant differences
785 between the mutants and parental accessions at $P_{fdr} < 0.05$.

Supporting information

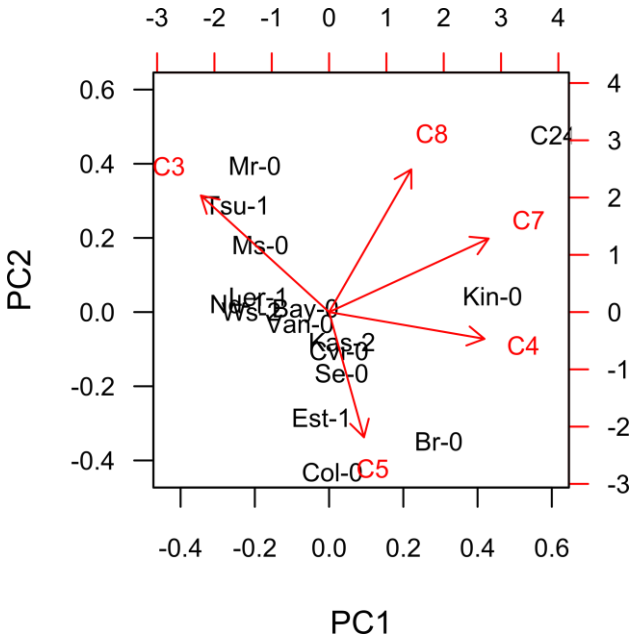
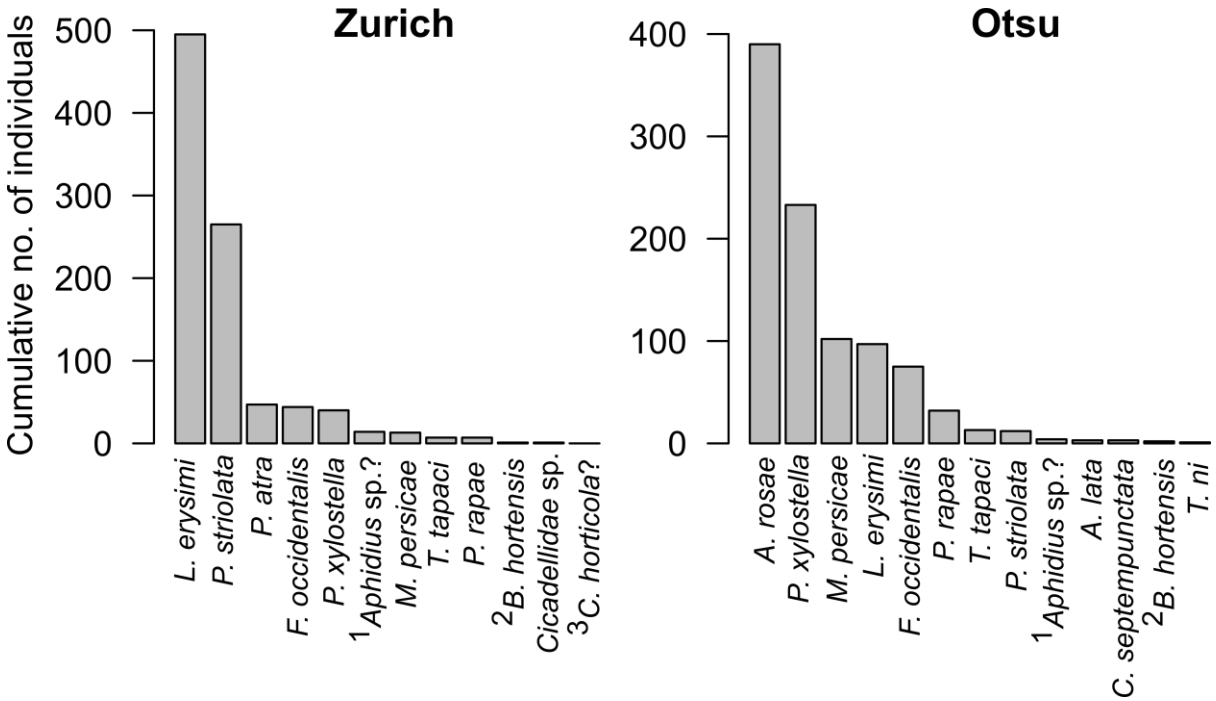


Figure S1. The first and second principal component (PC1 and PC2) summarizing the total amount (nmol/mg flesh weight) of C3-, C4-, C5-, C7-, and C8-Aliphatic glucosinolates for 17 accessions of *A. thaliana* (compiled from Chan et al. [34]). Arrows indicate contributions of each glucosinolate to PC1 and PC2.



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793 **Figure S2.** Cumulative number of each insect species in Zurich, Switzerland (left chart) and
794 Otsu, Japan (right chart) throughout the experiments. See Table 2 for the name of the
795 arthropod species. Notes: [1]Total number of parasitoid wasps and mummified aphids; [2]This
796 species is a non-insect arthropod; [3]Only a dwelling trace was observed.
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802 **Table S1.** Effects of trichome density, the first and second principal component (PC1 and PC2) of aliphatic glucosinolates (GLSs),
803 presence of flowering stem, and initial plant size on insect abundance and community composition among 17 natural accessions of
804 *Arabidopsis thaliana*.

Zurich

Response	Explanatory	Coef.	SE	*t*	P_{fdr}
Leaf holes	**Trichome**	**-0.53**	**0.10**	**-5.52**	**1.7.E-06**
	GLS_PC1	-0.15	0.08	-1.76	0.11
	GLS_PC2	-0.16	0.07	-2.44	0.06
	Flowering	-0.28	0.15	-1.89	0.24
	Initial leaf length	**0.45**	**0.08**	**5.98**	**1.9.E-07**
Diamond back moth	Trichome	-0.01	0.11	-0.14	0.89
	GLS_PC1	-0.20	0.09	-2.12	0.06
	GLS_PC2	0.00	0.07	0.05	0.99
	Flowering	0.21	0.17	1.24	0.45
	Initial leaf length	0.10	0.08	1.14	0.28
Striped flea beetle	**Trichome**	**-0.45**	**0.09**	**-5.10**	**6.0.E-06**
	GLS_PC1	-0.17	0.08	-2.15	0.06
	GLS_PC2	-0.13	0.06	-2.12	0.11

Otsu

Response	Explanatory	Coef.	SE	*t*	P_{fdr}
Turnip sawfly	**Trichome**	**-0.49**	**0.10**	**-4.72**	**0.00006**
	GLS_PC1	-0.08	0.09	-0.86	0.58
	GLS_PC2	**0.22**	**0.07**	**2.97**	**0.04**
	Flowering	0.24	0.16	1.53	0.15
	Initial leaf length	0.10	0.07	1.41	0.19
Diamond back moth	Trichome	0.11	0.11	0.97	0.47
	GLS_PC1	0.03	0.10	0.28	0.85
	GLS_PC2	0.06	0.08	0.78	0.71
	Flowering	0.10	0.17	0.58	0.56
	Initial leaf length	**0.34**	**0.08**	**4.25**	**0.00045**
Small cabbage white butterfly	**Trichome**	**-0.30**	**0.10**	**-2.99**	**0.01**
	GLS_PC1	0.09	0.08	1.08	0.57
	GLS_PC2	-0.04	0.07	-0.54	0.71

	Flowering	-0.06	0.14	-0.43	0.67		**Flowering**	**0.43**	**0.15**	**2.84**	**0.01**
	Initial leaf length	**0.37**	**0.07**	**5.18**	**2.7.E-06**		**Initial leaf length**	**0.22**	**0.07**	**3.06**	**0.01**
Turnip flea beetle	Trichome	-0.08	0.11	-0.76	0.68	Green peach aphid	Trichome	0.03	0.12	0.22	0.82
	GLS_PC1	-0.01	0.10	-0.08	0.93		GLS_PC1	-0.07	0.10	-0.69	0.66
	GLS_PC2	0.00	0.08	0.01	0.99		GLS_PC2	0.05	0.08	0.61	0.71
	Flowering	-0.40	0.17	-2.33	0.13		**Flowering**	**0.50**	**0.18**	**2.84**	**0.01**
	Initial leaf length	**0.23**	**0.09**	**2.63**	**0.01**		Initial leaf length	0.16	0.08	1.93	0.07
Mustard aphid	Trichome	-0.02	0.11	-0.20	0.89	Mustard aphid	Trichome	0.25	0.12	2.08	0.09
	GLS_PC1	-0.19	0.10	-1.93	0.08		GLS_PC1	0.15	0.10	1.42	0.47
	GLS_PC2	0.05	0.08	0.58	0.75		GLS_PC2	-0.02	0.09	-0.22	0.85
	Flowering	0.22	0.18	1.22	0.45		Flowering	0.27	0.18	1.44	0.17
	Initial leaf length	0.04	0.09	0.41	0.68		Initial leaf length	0.10	0.09	1.14	0.28
Western flower thrip	Trichome	0.12	0.11	1.10	0.54	Western flower thrip	Trichome	-0.10	0.11	-0.93	0.47
	GLS_PC1	-0.07	0.10	-0.67	0.55		GLS_PC1	-0.21	0.10	-2.21	0.34
	GLS_PC2	**0.30**	**0.08**	**3.86**	**0.002**		GLS_PC2	0.09	0.08	1.14	0.71
	Flowering	-0.51	0.18	-2.92	0.05		**Flowering**	**0.39**	**0.17**	**2.31**	**0.04**
	Initial Leaf Length	**0.37**	**0.09**	**4.23**	**7.0.E-05**		Initial leaf length	0.04	0.08	0.56	0.58
Leaf chewer	**Trichome**	**-0.42**	**0.09**	**-4.62**	**3.3.E-05**	Leaf chewer	**Trichome**	**-0.35**	**0.11**	**-3.18**	**0.01**
	GLS_PC1	**-0.21**	**0.08**	**-2.60**	**0.04**		GLS_PC1	-0.05	0.09	-0.57	0.68

	GLS_PC2	-0.10	0.06	-1.55	0.25		GLS_PC2	0.19	0.08	2.48	0.08
	Flowering	-0.14	0.14	-0.99	0.48		Flowering	0.31	0.17	1.84	0.09
	Initial Leaf Length	**0.41**	**0.07**	**5.67**	**4.2.E-07**		**Initial leaf length**	**0.31**	**0.08**	**3.94**	**0.0005**
Internal feeder	Trichome	0.04	0.11	0.32	0.89	Internal feeder	Trichome	0.10	0.11	0.93	0.47
	GLS_PC1	-0.21	0.10	-2.18	0.06		GLS_PC1	-0.09	0.09	-0.94	0.58
	GLS_PC2	0.15	0.08	1.93	0.13		GLS_PC2	0.04	0.08	0.54	0.71
	Flowering	0.13	0.17	0.78	0.58		**Flowering**	**0.70**	**0.17**	**4.10**	**0.0003**
	Initial leaf length	**0.21**	**0.09**	**2.41**	**0.02**		**Initial leaf length**	**0.18**	**0.08**	**2.21**	**0.05**
Specialist	**Trichome**	**-0.29**	**0.11**	**-2.75**	**0.02**	Specialist	Trichome	-0.25	0.11	-2.21	0.09
	GLS_PC1	**-0.28**	**0.09**	**-2.98**	**0.04**		GLS_PC1	0.01	0.10	0.10	0.92
	GLS_PC2	-0.05	0.07	-0.71	0.75		GLS_PC2	0.17	0.08	2.10	0.15
	Flowering	0.07	0.17	0.43	0.67		**Flowering**	**0.39**	**0.17**	**2.29**	**0.04**
	Initial leaf length	**0.29**	**0.08**	**3.46**	**0.001**		**Initial leaf length**	**0.32**	**0.08**	**3.98**	**0.0005**
Generalist	Trichome	0.09	0.11	0.78	0.68	Generalist	Trichome	0.03	0.11	0.26	0.82
	GLS_PC1	-0.13	0.10	-1.32	0.23		GLS_PC1	-0.18	0.09	-1.92	0.34
	GLS_PC2	**0.27**	**0.08**	**3.53**	**0.003**		GLS_PC2	0.06	0.08	0.81	0.71
	Flowering	-0.18	0.17	-1.04	0.48		**Flowering**	**0.65**	**0.16**	**3.93**	**0.0004**
	Initial leaf length	**0.40**	**0.09**	**4.59**	**2.2.E-05**		Initial leaf length	0.16	0.08	2.09	0.06
Species richness	Trichome	-0.14	0.10	-1.32	0.45	Species richness	Trichome	-0.14	0.11	-1.34	0.36

	GLS_PC1	**-0.24**	**0.09**	**-2.71**	**0.04**		GLS_PC1	-0.15	0.09	-1.69	0.37
	GLS_PC2	-0.04	0.07	-0.60	0.75		GLS_PC2	0.07	0.08	0.88	0.71
	Flowering	-0.09	0.16	-0.53	0.67		**Flowering**	**0.68**	**0.16**	**4.22**	**0.0003**
	Initial leaf length	**0.39**	**0.08**	**4.82**	**1.0.E-05**		**Initial leaf length**	**0.29**	**0.08**	**3.84**	**0.0005**
Shannon diversity	Trichome	-0.06	0.10	-0.61	0.72	Shannon diversity	Trichome	-0.09	0.10	-0.85	0.48
	GLS_PC1	-0.22	0.09	-2.40	0.05		GLS_PC1	-0.11	0.09	-1.25	0.51
	GLS_PC2	0.01	0.07	0.13	0.99		GLS_PC2	0.01	0.07	0.19	0.85
	Flowering	-0.21	0.16	-1.26	0.45		**Flowering**	**0.68**	**0.16**	**4.35**	**0.0003**
	Initial leaf length	**0.35**	**0.08**	**4.24**	**7.0.E-05**		**Initial leaf length**	**0.23**	**0.07**	**3.09**	**0.005**
Total abundance	**Trichome**	**-0.25**	**0.10**	**-2.43**	**0.02**	Total abundance	Trichome	-0.19	0.11	-1.72	0.09
	GLS_PC1	**-0.28**	**0.09**	**-3.11**	**0.002**		GLS_PC1	-0.05	0.09	-0.54	0.59
	GLS_PC2	0.00	0.07	-0.04	0.97		**GLS_PC2**	**0.20**	**0.08**	**2.58**	**0.01**
	Flowering	0.04	0.16	0.24	0.81		**Flowering**	**0.59**	**0.17**	**3.55**	**0.001**
	Initial leaf length	**0.36**	**0.08**	**4.37**	**2.3.E-05**		**Initial leaf length**	**0.34**	**0.08**	**4.39**	**0.00002**

Standardized coefficient (Coef.), standard error (SE), *t*-value, and FDR-corrected *P*-value are shown for each explanatory variable in Zurich, Switzerland and Otsu, Japan. Bold values highlight significant effects at $P_{fdr} < 0.05$.

Table S2. Effects of trichome density, presence of flowering stem, and initial plant size on insect abundance and community composition in a comparison between glabrous mutants and their parental accessions.

Zurich

Response	Explanatory	Coef.	SE	*t*	P_{fdr}
Leaf holes	**Trichome**	**-0.42**	**0.14**	**-3.05**	**0.027**
	Flowering	**-1.18**	**0.27**	**-4.40**	**0.001**
	Initial leaf length	**0.42**	**0.13**	**3.14**	**0.021**
Leaf chewer	Trichome	-0.06	0.17	-0.33	0.959
	Flowering	-0.19	0.33	-0.57	0.812
	Initial leaf length	0.39	0.16	2.38	0.062
Specialist	Trichome	0.01	0.17	0.05	0.959
	Flowering	0.23	0.33	0.69	0.812
	Initial leaf length	0.32	0.16	1.95	0.068
Species richness	Trichome	0.01	0.17	0.05	0.959
	Flowering	0.08	0.34	0.24	0.812
	Initial leaf length	0.32	0.17	1.89	0.068
Shannon diversity	Trichome	-0.16	0.16	-0.95	0.959
	Flowering	-0.13	0.32	-0.41	0.812
	Initial leaf length	0.36	0.16	2.25	0.062

Otsu

Response	Explanatory	Coef.	SE	*t*	P_{fdr}
Turnip sawfly	**Trichome**	**-0.57**	**0.15**	**-3.94**	**0.002**
	Flowering	-0.42	0.29	-1.46	0.397
	Initial leaf length	0.23	0.16	1.44	0.318
Leaf chewer	**Trichome**	**-0.47**	**0.15**	**-3.07**	**0.009**
	Flowering	-0.43	0.30	-1.40	0.397
	Initial leaf length	0.26	0.17	1.55	0.318
Specialist	**Trichome**	**-0.49**	**0.15**	**-3.16**	**0.009**
	Flowering	-0.40	0.30	-1.31	0.397
	Initial leaf length	0.26	0.17	1.58	0.318
Species richness	Trichome	-0.24	0.17	-1.37	0.214
	Flowering	0.12	0.34	0.36	0.722
	Initial leaf length	0.08	0.19	0.40	0.689
Shannon diversity	Trichome	-0.17	0.18	-0.97	0.339
	Flowering	0.14	0.35	0.40	0.722
	Initial leaf length	0.13	0.19	0.71	0.582

Total abundance	Trichome	0.04	0.17	0.22	0.959	Total abundance	**Trichome**	**-0.46**	**0.16**	**-2.86**	**0.011**
	Flowering	0.30	0.33	0.91	0.812		Flowering	-0.16	0.32	-0.52	0.722
	Initial leaf length	0.33	0.16	2.00	0.068		Initial leaf length	0.18	0.17	1.03	0.468

Standardized coefficient (Coef.), standard error (SE), *t*-value, and FDR-corrected *P*-value are shown for each explanatory variable. Bold values highlight significant effects at $P_{fdr} < 0.05$. NA means no data available. The trichome density presents differences between the null and hypomorphic mutants. Because the trichome density had a significant effect on flea beetles and sawflies (Table S1), we focused on species, guild, and community indices comprising these two leaf chewers.

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