

1 Running head: Tests of associational defence in *Arabidopsis*

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3 **Tests of associational defence provided by hairy plants for glabrous plants of**  
4 ***Arabidopsis halleri* subsp. *gemmaifera* against insect herbivores**

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21 **Abstract**

22 1. We investigated trichome-producing (hairy) and trichomeless (glabrous) plants of  
23 *Arabidopsis halleri* subsp. *gemmifera* to test whether plant resistance to herbivory depends on  
24 the plants' phenotypes and/or the phenotypes of neighbouring plants (associational effects).

25 2. We conducted a common garden experiment in which the relative frequency of hairy and  
26 glabrous plants was manipulated. Two species of leaf-chewing insects (larvae of a white  
27 butterfly and a cabbage sawfly) were found less often on hairy plants than on glabrous plants.  
28 In contrast, the numbers of aphids and flea beetles did not differ significantly between hairy  
29 and glabrous plants. For none of these insects did abundance depend on the frequency of the  
30 two plant morphs.

31 3. A field survey was conducted in two natural populations of *A. halleri*. In the first  
32 population, a species of white butterfly was the dominant herbivore, and hairy plants incurred  
33 less leaf damage than glabrous plants across two years. In contrast, in the other population,  
34 where flea beetles were dominant, there were no consistent differences in leaf damage  
35 between the two types of plants. In neither of the two populations did we find evidence for  
36 associational effects.

37 4. This study did not provide any conclusive evidence of associational effects of anti-  
38 herbivore resistance, but we did find that trichomes can confer resistance to certain  
39 herbivores. Given the results of our previous work on associational effects against a flightless  
40 leaf beetle, such associational effects of the trichome dimorphism of *A. halleri* were  
41 herbivore-specific. [243 up to 250 words]

42 Key words: Anti-herbivore resistance, *Arabidopsis halleri* subsp. *gemmifera*, Associational  
43 effects, Polymorphism, Trichome

## 44 **Introduction**

45 As sessile organisms, plants are consumed by a diverse array of insect herbivores (Strong *et*  
46 *al.*, 1984; Lewinsohn *et al.*, 2005). Thus, plants developed various resistance traits to protect  
47 themselves from herbivory (Schoonhoven *et al.*, 2005). For example, trichomes (plant  
48 epidermal hairs) are considered a physical resistance trait against herbivory (Levin, 1973;  
49 Hanley *et al.*, 2007). There is increasing evidence that trichomes alter the feeding efficiency  
50 and oviposition preferences of various herbivores, including lepidopterans and coleopterans  
51 (e.g. Zvereva *et al.*, 1998; Reymond *et al.*, 2004; Handley *et al.*, 2005; Sletvold *et al.*, 2010;  
52 Yamawo *et al.*, 2012). Plant trichomes are also known to show large phenotypic variation  
53 that is governed genetically (Ågren & Schemske, 1994; Kivimäki *et al.*, 2007; Bloomer *et al.*,  
54 2012). Although trichomes exhibit quantitative phenotypic variation in their density or  
55 number (Ågren & Schemske, 1994), the presence/absence of trichomes (i.e. trichome-  
56 producing and trichomeless plants) is sometimes regulated by a few major genes (Kivimäki *et*  
57 *al.*, 2007; Kawagoe *et al.*, 2011; Bloomer *et al.*, 2012).

58           When anti-herbivore resistance traits are sufficiently varied within a plant  
59 community or population, the amount of herbivory on an individual plant depends not only  
60 on the plant's own phenotype but also on the neighbouring plant types (Atsatt & O'Dowd,  
61 1976; reviewed by Agrawal *et al.*, 2006; Barbosa *et al.*, 2009). This phenomenon is referred  
62 to as an associational effect and includes any consumer effect mediated by the composition of  
63 plant types within a neighbourhood on a focal individual plant (Underwood *et al.*, 2014). For  
64 example, palatable plants benefit from the presence of unpalatable plants via associational  
65 resistance if the latter repels herbivores away from the nearby palatable plants (Tahvanainen  
66 & Root, 1972). In contrast, unpalatable plants suffer from associational susceptibility when  
67 herbivores settle on palatable plants and then disperse onto unpalatable ones (White &

68 Whitham, 2000). Associational effects are predicted to influence plant diversity, because they  
69 may regulate population growth of multiple plant types in a density- or frequency-dependent  
70 manner (Hambäck *et al.*, 2014).

71 To date, associational effects have been mostly investigated as a type of  
72 interspecific interaction (e.g. Tahvanainen & Root, 1972; White & Whitham, 2000; Le Guigo  
73 *et al.*, 2012), but they can also be examined through the lens of intraspecific variation of anti-  
74 herbivore resistance traits (Wise *et al.*, 2009; Hambäck *et al.*, 2009). Several studies of  
75 cultivated plants have documented associational effects among resistant and susceptible  
76 genotypes within a plant species (Cantero & Sanford, 1984; Hambäck *et al.*, 2009). In these  
77 cases, a focal plant genotype may benefit via associational effects from the increasing  
78 proportion of another genotype in close vicinity. These interactions lead to frequency  
79 dependence in herbivory, which can promote the coexistence of different genotypes if rare  
80 genotypes are protected from herbivory (Wise *et al.*, 2009; Underwood *et al.*, 2014). Little is  
81 known about associational effects and their frequency dependence with respect to natural  
82 variation in anti-herbivore resistance traits within a plant species.

83 *Arabidopsis halleri* (L.) O’Kane & Al-Shehbaz subsp. *gemmaifera* (Matsum.)  
84 O’Kane & Al-Shehbaz [Brassicaceae/Cruciferae] has both trichome-producing (referred to  
85 hereafter as hairy) and trichomeless (glabrous) plants. The presence or absence of trichomes  
86 is associated with the allelic status of a candidate gene (Kawagoe *et al.*, 2011). Our previous  
87 study found that leaf damage on *A. halleri* depended on the relative frequency of  
88 neighbouring hairy and glabrous plants in a natural population where plants were heavily  
89 infested by an oligophagous leaf beetle, *Phaedon brassicae* Baly [Coleoptera:  
90 Chrysomelidae] (Sato *et al.*, 2014). In a choice experiment, we also revealed that when hairy  
91 plants were rare, they incurred less herbivory by *P. brassicae* than did glabrous plants,

92 whereas when hairy plants were common, they were not resistant to herbivory (Sato *et al.*,  
93 2014). However, white butterflies, flea beetles, moths, and aphids can also be observed in  
94 natural populations of *A. halleri* (Y. Sato, personal observation), and the dominant herbivores  
95 vary even between closely located populations (Kawagoe & Kudoh, 2010). It is thus possible  
96 that patterns of associational effects depend on the herbivore species on the trichome  
97 dimorphism of *A. halleri*. Here, we examined whether such associational effects exist against  
98 herbivores other than the leaf beetle, *P. brassicae*.

99           In this study, two specific questions were addressed: (1) Does trichome production  
100 act as an anti-herbivore resistance trait, and if so, to which herbivores does it provides  
101 resistance? (2) Do any of the anti-herbivore effects of trichomes depend on the relative  
102 frequencies of hairy and glabrous plants? We performed both an experimental and an  
103 observational study to answer these questions. Firstly, we manipulated the relative frequency  
104 of hairy and glabrous plants in a common garden to test the resistance role of trichomes and  
105 their associational effects against herbivores. Secondly, we conducted a series of field  
106 surveys under different herbivore community structures to examine whether similar patterns  
107 of herbivory occurred in natural populations.

108

## 109 **Materials and Methods**

110

### 111 *Study system*

112 *Arabidopsis halleri* subsp. *gemmaifera* is a perennial herb distributed across Japan and the  
113 Russian Far East (Al-Shehbaz & O’Kane, 2002). In the lowlands of western Japan, self-  
114 incompatible flowers start to bloom in late March or early April, and fruit set is ca. 80%

115 unless the flowers are consumed by herbivores (Kawagoe & Kudoh, 2010). After flowering,  
116 plants produce new rosettes on the main and axillary meristems of flowering stems, and these  
117 rosettes often establish as clonal offspring once they have rooted and attached themselves to  
118 the ground. Hairy and glabrous plants co-occur in a natural population in central Japan  
119 (Kawagoe *et al.*, 2011). The hairy and glabrous phenotypes in this population are associated  
120 with allelic variation in a candidate gene, *GLABROUS1* (*GL1*) but are not associated with  
121 other genes (Kawagoe *et al.*, 2011; see Grebe, 2012 for a review of *GL1* in *A. thaliana*).  
122 Hairy plants develop trichomes on the surfaces of their leaves and stems, but not on the  
123 flowers and fruits, whereas glabrous plants have no trichomes except along the leaf margin.  
124 The two morphs have no other apparent morphological differences. Hairy plants produced  
125 fewer fruits than glabrous plants under weak herbivory (Kawagoe *et al.*, 2011), indicating a  
126 trade-off with trichome production. Glucosinolate profiles were not associated with either the  
127 hairy or the glabrous phenotype in our seed source population, as described below (Sato *et al.*,  
128 2014).

129

### 130 *Common garden experiment*

131 Mature fruits of *A. halleri* were collected from a natural population located in Taka-cho,  
132 Hyogo Prefecture, central Japan (35°06'N, 134°56'E, ca. 200 m in altitude, Sato *et al.*, 2014).  
133 The fruits were harvested from 14 maternal plants (including seven hairy and seven glabrous  
134 plants) in early July 2011; the maternal plants were spaced at least 5 m apart to minimize the  
135 likelihood of multiple samples being taken from a single clone. These fruits provided the  
136 seeds from which the plants in our experiment were grown.

137           For the common garden experiment, we initially prepared ca. 200 plants in an  
138 indoor space that contained no herbivores. Fifty seeds from each maternal family were sown

139 on a petri dish (diameter 9 cm, depth 1.5 cm) filled with moistened quartz sand on 15 August  
140 2011 and were allowed to germinate at room temperature. Three seedlings per family were  
141 transplanted to a plastic pot (diameter 10.5 cm, depth 9 cm) filled with mixed soil  
142 (pumice:leaf mold:peat moss = 1:1:1) on 25 November 2011. All seedlings had two to four  
143 leaves when they were transplanted. To prevent herbivory, these plants were placed inside  
144 transparent plastic cases (75 cm × 45 cm with a depth of 18 cm, 28 pots per case). The plants  
145 grew for four months under natural sunlight (10–12 h in day length) with average daily  
146 temperatures of 10–30 °C. The location of the cases was rotated every month. All plants were  
147 transplanted individually into plastic pots about a month before the start of the experiment to  
148 avoid competition within the cases. The liquid fertiliser Hyponex (Hyponex, N:P:K = 6:10:5,  
149 Hyponex Japan, Osaka, Japan) was diluted 1000-fold and supplied monthly until the  
150 beginning of the experiment. Photosynthetically active radiation was 800–1100 and 100–300  
151  $\mu\text{mol}/\text{m}^2/\text{s}$  on a sunny and cloudy day, respectively. The red:far-red ratio ranged from 1.0 to  
152 1.3 (LI-190 Quantum Sensor, LI-COR, Lincoln, NE, USA).

153           The potted plants were transferred to the experimental garden of the Center for  
154 Ecological Research, Kyoto University (34°58'N, 135°57'E, ca. 100 m in altitude) on 28  
155 March 2012. All cultivated plants were sorted in ascending order of leaf length (the length of  
156 the longest radical leaf, which ranged from 33 to 71 mm), and the largest 150 plants from the  
157 14 maternal families were used in the experiments, in order to ensure that similarly sized  
158 plants were used. We controlled for plant size to avoid confounding effects on herbivore  
159 abundance per plant.

160           Twenty-five individuals were arranged squarely in each of six 1 × 1 m plots filled  
161 with fine gravel. We established two treatments for the frequency of hairy and glabrous  
162 plants within a plot (Fig. 1); three plots consisted of 21 hairy and four glabrous plants

163 (referred to as hairy-abundant plots), and the remaining three consisted of four hairy and 21  
164 glabrous plants (referred to as glabrous-abundant plots). The plots were spaced 1.7 m apart.  
165 The hairy or glabrous plants of similar size (< 11 mm difference in the length of the longest  
166 radical leaf within a single plot) were placed in the centre of the plot, while plants of random  
167 size occupied the positions at the edge (Fig. 1).

168           Herbivore abundance and plant performance were monitored weekly on sunny days  
169 from April to June 2012 (2, 9, 17, 25, and 30 April; 7, 14, 21, and 28 May; 4, 11, 18, and 25  
170 June). The mustard aphid *Lipaphis erysimi* Kaltentbach [Hemiptera: Aphididae] and green  
171 peach aphid *Myzus persicae* Sulzer [Hemiptera: Aphididae] occurred on *A. halleri* at our  
172 experimental site (see Supporting information, Fig. S1). The small cabbage white butterfly  
173 *Pieris rapae* L. [Lepidoptera: Pieridae], cabbage sawfly *Athalia infumata* Marlatt  
174 [Hymenoptera: Tenthredinidae], and flea beetle *Phyllotreta striolata* Fabricius [Coleoptera:  
175 Chrysomelidae] also occurred, mainly after the flowering season (Table 1; Fig. S1). Wingless  
176 and winged aphids were counted separately, as the former represented a growing colony  
177 while the latter was likely to be associated with dispersal events. We counted *L. erysimi* and  
178 *M. persicae* as a single group because the two species were difficult to distinguish accurately  
179 in the field due to their similar colours and morphology at the nymph stage. In addition, we  
180 recorded the number of mummies (i.e. aphids fed on by parasitoid wasps) after the peak  
181 abundance of wingless aphids (14 May 2012). The numbers of intact and damaged leaves  
182 were recorded separately in order to distinctly evaluate plant growth and herbivory damage.  
183 The numbers of flowers and mature fruits were counted weekly for each plant. We counted  
184 flowers as the number of pedicels, including both fruited and non-fruited flowers, to estimate  
185 the flower production of each plant throughout the season. The seed production of each plant  
186 could not be evaluated because mature seeds spontaneously dropped; therefore, we used the  
187 number of mature fruits to estimate seed production. A mature fruit contained  $8.3 \pm 2.2$  seeds

188 under our experimental conditions (mean  $\pm$  SD,  $n = 19$  fruits). To avoid edge effects in the  
189 plots, we analysed the nine plants in the centre of each plot (Fig. 1). Three edge plants died  
190 from unknown causes (not related to herbivory levels). All measurements were carried out  
191 between 8:00 and 17:00.

192

### 193 *Field survey*

194 We conducted field surveys in two natural populations of *A. halleri*; Ojigahata, Shiga  
195 (35°12'N, 136°23'E, ca. 300 m in altitude) and Minoh, Osaka, Japan (34°50'N, 135°28'E, ca.  
196 50 m in altitude). The two sites were selected because the green-veined white butterflies,  
197 *Pieris napi* L. [Lepidoptera: Pieridae], were predominantly observed at one site (Ojigahata)  
198 while the flea beetles *Phyllotreta striolata* were predominantly observed at the other site  
199 (Minoh). Both sites were located in gravel areas near roads that were covered with sparse  
200 vegetation. The population size and proportion of hairy plants were similar between the two  
201 sites (no. of plants = 500–1000, percentage of hairy plants = 40–60%). We observed other  
202 cruciferous plants (*Cardamine hirsuta*, *C. impatiens*, *C. leucantha*, and *C. scutata*) at these  
203 sites, but they occurred only rarely.

204 Field surveys were conducted twice a year in spring (late May or early June) and  
205 autumn (late September or early October), as *P. napi* shows bivoltinism (Fukuda *et al.*, 1984).  
206 For each population and census, we recorded the trichome phenotype (hairy or glabrous) and  
207 the proportion of leaf area lost to herbivory for all individual plants in randomly chosen  
208 circular patches (1 m in diameter). Our preliminary survey confirmed that the number of  
209 plants within each circular patch began to plateau as the patch size increased. At the  
210 Ojigahata site,  $2.92 \pm 0.25$ ,  $5.52 \pm 0.70$ , and  $7.40 \pm 1.02$  plants occurred within 0.5-, 1.0-, and  
211 3.0-m-diameter patches, respectively (mean  $\pm$  SE,  $n = 25$  patches examined). At the Minoh

212 site,  $3.71 \pm 0.29$ ,  $7.68 \pm 0.61$ , and  $10.68 \pm 0.95$  plants occurred within 0.5-, 1.0-, and 3.0-m-  
213 diameter patches, respectively (mean  $\pm$  SE,  $n = 28$  patches). Therefore, we focused on the  
214 local interaction in 1-m-diameter patches. We examined 70–90 patches, including 150–500  
215 hairy and 200–550 glabrous plants, at the Ojigahata site; and we examined 65–85 patches,  
216 including 180–350 hairy and 290–380 glabrous plants, at the Minoh site (see also Table 2 and  
217 3 for detailed survey dates and sample sizes). The proportion of leaf area lost to herbivory  
218 (referred to hereafter as the leaf damage) was evaluated visually and recorded as one of 11  
219 successive values: 0 (no damage), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 (complete  
220 leaf loss). In addition, to evaluate to what extent our method of quantifying the leaf damage  
221 reflected the intensity of herbivory, we also recorded the number of intact and damaged  
222 leaves of 19 plants at the Ojigahata site. This additional measurement confirmed that the leaf  
223 damage estimated by our method was highly correlated with the total proportion of leaves  
224 damaged (Pearson’s product moment correlation; both variables were arcsine-transformed,  $r$   
225  $= 0.92$ ,  $t_{17} = 9.4$ ,  $P < 0.0001$ ). We also identified and counted herbivorous insects within the  
226 study area during the survey. All observations and surveys were carried out for 4–6 hours  
227 between 9:00 and 16:00.

228

### 229 *Statistical analysis*

230 We used the data collected in our experiment to analyse the effects of the trichome phenotype  
231 (hairy or glabrous phenotype for each plant) and frequency condition (hairy-abundant or  
232 glabrous-abundant for each plot) as fixed effects explaining the number of herbivores,  
233 flowers, or leaves per plant (response variables). The interaction term of the trichome  
234 phenotype  $\times$  frequency condition was incorporated as a fixed effect to determine whether the  
235 main effects of the trichome phenotype on the herbivore abundance or plant performance

236 depended on the frequency condition (namely, associational effects). To analyse the count  
237 response, we used generalised linear mixed models (GLMMs: Bolker *et al.*, 2009) with a  
238 Poisson error structure and a log link function. The significance levels of the main effects  
239 were first analysed using likelihood ratio tests. Then, the trichome-by-frequency interaction  
240 was analysed to test associational effects. The cumulative number of each herbivore per plant  
241 was analysed as the response variable to reflect both herbivory intensity and duration for  
242 plants (i.e. herbivory load: Ruppel, 1983; Fournier *et al.*, 2005). As an exception, the number  
243 of mummified aphids was analysed using the abundance that was recorded on 28 May  
244 because the mummified aphids were attached to plant surfaces and reflected the accumulated  
245 number by themselves.

246         Aphids were observed on leaves throughout the study, whereas white butterflies,  
247 cabbage sawflies, and flea beetles were observed only during June (Fig. S1). Thus, to exclude  
248 the direct confounding interactions of the late-coming herbivores, we separately analysed the  
249 cumulative number of wingless and winged aphids counted per plant before 28 May. To  
250 examine plant fecundity and growth corresponding to this period, we analysed the data  
251 collected until the end of May regarding the number of flowers and intact leaves. The number  
252 of mature fruits was also analysed; however, the result is not presented in the main text  
253 because it followed a similar pattern to that of the number of flowers during the early period  
254 of the experiment (Fig. S2) and no significant effects were detected regarding the number of  
255 fruits (see Table 1 and Results section below). A small number of leaves (median = 0 leaves)  
256 were damaged in the period before 28 May 2012 (Fig. S2); therefore, the data for this  
257 variable during the early period were not analysed.

258         For the late-coming herbivores (white butterflies, sawflies, and flea beetles), we  
259 analysed the cumulative number of eggs and larvae of *P. rapae*, larvae of *A. infumata*, and

260 adults of *P. striolata*, in which the cumulative number of each herbivore was calculated  
261 throughout the study. Then, to examine the effects of the late-coming herbivores on plant  
262 growth, we analysed the number of damaged leaves at the end of the study (25 June 2012).  
263 Many leaves were damaged by the end of the experiment (Fig. S2); thus, their number was  
264 sufficient to estimate the degree of herbivory on the two types of plants. The number of  
265 mature fruits during the late season was not analysed because the impact of these late-coming  
266 insects was so severe that a large proportion of the fruits was lost to herbivory.

267           In some of the GLMM analyses, we included an offset term of  $\log_e(x + 1)$  to  
268 examine the rates of the count response (Nelder & Frome, 1984). This method allows us to  
269 handle the effects of sampling effort, or total counts, as a covariate without estimating a slope  
270 coefficient in the analysis of the count response. For the analyses of winged and mummified  
271 aphids, we included the number of wingless aphids as an offset term to consider the density-  
272 dependent dispersal or parasitism on living aphids. The number of flowers was included as an  
273 offset term to analyse fruit set (that is, the proportion of mature fruits in the total number of  
274 flowers produced) for the number of mature fruits. To analyse the number of damaged leaves,  
275 we included the number of intact leaves as an offset term to evaluate the per capita leaf  
276 damage.

277           We incorporated two random effects into all of the GLMMs above. Firstly, to  
278 reflect the variance of herbivore abundance and plant performance among the plots in the  
279 analyses, we included the plot ID as a random effect. Secondly, because we designed the  
280 experiment to control for plant size, the number of individual plants differed between  
281 maternal families (mean  $\pm$  SD =  $4.2 \pm 2.8$ ,  $n = 14$  maternal plants). Thus, the seed plant ID  
282 was incorporated as another random effect to consider the potential variation in other plant  
283 traits related to the maternal families.

284 We used the field survey data to analyse the effects of the trichome phenotype, the  
285 proportion of glabrous plants in a patch (which represents the relative frequency of the two  
286 plant types), and the total number of plants (which represents the density of *A. halleri* plants)  
287 as fixed effects explaining the leaf damage. Leaf damage was analysed using linear mixed  
288 models, in which the response variable was arcsine-square root transformed to improve the  
289 normality of residuals. To test whether the effect of the trichome phenotype on leaf damage  
290 depended on the proportion of glabrous plants (i.e. associational effects), we also analysed  
291 the interaction between the trichome phenotype and the proportion of glabrous plants in a  
292 patch as another fixed effect. The patch ID was incorporated as a random effect so that  
293 multiple individuals in a patch were not treated as independent samples. We used likelihood  
294 ratio tests to analyse the significance of each fixed effect, in which the main effects were first  
295 tested and then the interaction term was examined. Furthermore, in the linear mixed models,  
296 we estimated coefficients of the terms ‘the proportion of glabrous plants in a patch’ and ‘the  
297 total number of *A. halleri* in a patch’ to examine the sign and magnitude of the effects of the  
298 frequency of hairy and glabrous plants and their density on leaf damage. When the  
299 coefficients significantly deviated from zero, we added trend lines to figures using single  
300 regression of the leaf damage on the proportion of glabrous plants in a patch. These analyses  
301 were separately performed for each survey at the Ojigahata and Minoh sites.

302 All statistical analyses were performed using R version 2.15.0 (R Development  
303 Core Team, 2012). We used the `glmer` and `lmer` functions (in the `lme4` package: Bates *et al.*,  
304 2012) for GLMMs and linear mixed model analyses. We chose the maximum likelihood  
305 method for the `lmer` function and the Laplace approximation for the `glmer` function to  
306 estimate likelihoods and coefficients.

307

308 **Results**

309

310 *Common garden experiment*

311 Aphids emerged in late April, followed by *P. rapae*, *A. infumata*, and *P. striolata* (Fig. S1).  
312 We also observed a few *Thrips palmi* Karny [Thysanoptera: Thripidae] and *Plutella*  
313 *xylostella* L. [Lepidoptera: Yponomeutidae] on *A. halleri* during June, but their abundance  
314 was not recorded because they were rare. Three of the 240 *P. rapae* larvae counted in this  
315 study were attacked by parasitoid wasps. Although we counted aphids as a single herbivore  
316 group, detailed identification under a binocular microscope on 30 April 2012 revealed that 67  
317 specimen samples contained 24 individuals of *L. erysimi* and 43 individuals of *M. persicae*.

318           During the early period, the number of wingless aphids did not differ between hairy  
319 and glabrous plants (Fig. 2a: Table 1), indicating little resistance role of the trichome  
320 production against the aphids. Wingless aphids occurred slightly more often on hairy plants  
321 under the glabrous-abundant condition (the trichome  $\times$  frequency interaction,  $P < 0.05$ : Table  
322 1; Fig. 2a). The number of winged aphids was not significantly affected by the trichome  
323 phenotype (Table 1; Fig. 2b). Like the wingless aphids, mummified aphids occurred more  
324 often on hairy plants under the glabrous-abundant condition (Table 1; Fig. 2c). During this  
325 early period, glabrous plants showed a higher flower production than hairy plants (the main  
326 effect of the trichome phenotype,  $P < 0.05$ : Table 1; Fig. 2d). The fruit production was not  
327 significantly affected by the trichome phenotype, frequency condition, and their interaction  
328 (Table 1; Fig. S2). The number of intact leaves did not differ between hairy and glabrous  
329 plants (the main effect of the trichome phenotype,  $P = 0.68$ : Table 1), but glabrous plants had  
330 a larger number of intact leaves than hairy ones under the glabrous-abundant condition (the  
331 trichome  $\times$  frequency interaction,  $P < 0.05$ : Table 1; Fig. 2e).

332           During the later period of the experiment, *P. rapae* eggs, *P. rapae* larvae, and *A.*  
333 *infumata* larvae tended to occur less often on hairy plants within the frequency condition (the  
334 main effect of the trichome phenotype,  $P < 0.05$ ,  $P = 0.07$ , and  $P = 0.42$  for *Pi. rapae* eggs, *P.*  
335 *rapae* larvae, and *A. infumata* larvae, respectively: Table 1; Figs. 2f, g, h), providing evidence  
336 for a resistance role of the trichome production against these herbivores. The number of *P.*  
337 *rapae* and *A. infumata*, was not interactively affected by the trichome phenotype and  
338 frequency condition (Table 1), indicating no associational effects. Adults of the flea beetle, *P.*  
339 *striolata*, occurred slightly more frequently on hairy plants (the main effect of the trichome  
340 phenotype,  $P = 0.07$ : Fig. 2i), but there was no evidence for associational effects (i.e. no  
341 significant trichome  $\times$  frequency interaction: Table 1). During this later period, hairy plants  
342 tended to have fewer damaged leaves than did glabrous plants (Fig. 2j), although the main  
343 effect of the trichome phenotype was not significant (Table 1).

344

#### 345 *Field survey*

346 The green-veined white butterfly, *Pieris napi*, was a major insect herbivore in the Ojigahata  
347 site, whereas the flea beetle, *Phyllotreta striolata*, was dominant in the Minoh site (Table 2a).  
348 On 15 September 2012 at the Ojigahata site, we found one egg on a hairy plant and eight  
349 eggs on glabrous plants. On 27 May 2013 at the Ojigahata site, two eggs were found on  
350 glabrous plants and we observed a larva of *P. napi*. Larvae of *A. infumata* were found  
351 occasionally at the Ojigahata and Minoh site (Table 2a). We did not observe any parasitoids  
352 attacking herbivorous insects in the two populations.

353           There was less leaf damage on hairy plants than on glabrous plants over the course  
354 of two years at the Ojigahata site (the main effect of the trichome phenotype,  $P < 0.05$  over  
355 the four surveys: Table 2; Fig. 3a). On the other hand, no consistently significant patterns

356 were observed between the leaf damage on hairy and glabrous plants over the course of two  
357 years at the Minoh site (Table 2; Fig. 3b). In these two populations, we rarely found  
358 significant interactions (2 out of 16 cases) between the trichome phenotype and the  
359 proportion of glabrous plants in a patch (except for 15 September 2012 and 26 May 2013 at  
360 the Ojigahata site,  $P < 0.05$ : Table 2), indicating little associational effects on the leaf damage.  
361 The proportion of glabrous plants in a patch was not significantly related to leaf damage in 13  
362 out of 16 cases (except for one case at the Ojigahata site and two cases at the Minoh site:  
363 Table 3; Fig. 4). The total number of *A. halleri* in a patch showed few or negative effects  
364 from leaf damage; six out of 16 cases were significantly negative (two cases at the Ojigahata  
365 site and four cases at the Minoh site: Table 3).

366

## 367 **Discussion**

368 In this study, we were unable to detect any conclusive evidence of associational  
369 effects; however, we did find evidence to suggest a role for trichomes as an anti-herbivore  
370 resistance trait against certain herbivores. In the common garden experiment, we observed  
371 fewer eggs and larvae of the small cabbage white, *Pieris rapae*, on hairy plants than on  
372 glabrous plants. We also found that larvae of the cabbage sawfly, *Athalia infumata*, tended to  
373 occur less often on hairy plants than on glabrous plants. During the later period when these  
374 two species of leaf chewers were abundant, hairy plants tended to suffer less leaf damage  
375 than glabrous plants. In contrast, we did not observe a reduced abundance of aphids (*Myzus*  
376 *persicae* and *Lipaphis erysimi*) and flea beetles (*Phyllotreta striolata*) on hairy plants in the  
377 common garden. In the field surveys, hairy plants incurred slightly less herbivory than  
378 glabrous plants in a natural population where the green-veined white butterfly, *Pieris napi*,  
379 was dominant. In contrast, no consistent differences in leaf damage were detected between

380 hairy and glabrous plants in the other population where *P. napi* was rarely observed and  
381 where the flea beetle was dominant.

382           Anti-herbivore resistance functions of trichomes have been reported for interactions  
383 between other *Arabidopsis* trichomes and leaf-chewing insects. Leaf trichomes are known to  
384 prevent damage by the diamondback moth *Plutella xylostella* in *A. lyrata* (Sletvold *et al.*,  
385 2010) and to reduce the performance of *P. rapae* larvae in *A. thaliana* (Reymond *et al.*, 2004;  
386 reviewed by van Poecke, 2007). In addition, in *A. halleri*, our previous study revealed that  
387 trichome production influenced the feeding preferences of adults and reduced the  
388 performance of larvae of the leaf beetle *Phaedon brassicae* (Sato *et al.*, 2014). Given the  
389 common feeding habit among the white butterflies and sawflies (which tended to occur less  
390 often on hairy plants in the common garden) in addition to the leaf beetles, our findings  
391 suggest a role for trichomes in resistance against leaf chewers (Table 4). In *A. thaliana* and *A.*  
392 *lyrata*, trichomes are also known to repel oviposition by *P. xylostella* (Handley *et al.*, 2005;  
393 Sletvold *et al.*, 2010). The result that fewer eggs of *P. rapae* were observed on hairy plants in  
394 our common garden experiment may support such negative effects of trichomes on the  
395 oviposition preference of lepidopterans.

396           Plant trichomes are not always effective as a mechanism of herbivore resistance.  
397 Their effects depend on herbivore feeding habits (Andres & Conner, 2003), herbivore life  
398 stage (Yamawo *et al.*, 2012), and plant ontogeny (Puentes & Ågren, 2013). For instance, in a  
399 community-level study of manzanita (*Arctostaphylos*) species, Andres and Conner (2003)  
400 suggested that trichomes were ineffective as a resistance trait against small or sedentary  
401 herbivores because such herbivores have less contact with plant hairs during their life cycles.  
402 In our study, aphids infrequently contacted *A. halleri* trichomes because the flowering buds  
403 of *A. halleri* did not produce trichomes, and many of the aphids were observed on the tops of

404 flowering stems (Y. Sato, personal observation). In addition, young leaves of hairy plants  
405 produce ca. 60 trichomes/cm<sup>2</sup>, but the trichome density becomes low (ca. 10 trichomes/cm<sup>2</sup>)  
406 in mature leaves (Y. Sato, unpublished data). Thus, flea beetle (*Phyllotreta striolata*) adults,  
407 which have a body size of a few millimetres, contacted few hairs at such a sparse trichome  
408 density. Furthermore, adult flea beetles can move from one leaf to another by jumping, so  
409 trichomes are unlikely to interfere with their mobility (Table 4). The potential role of  
410 trichomes in resistance should be tested with regards to plant ontogeny in order to fully  
411 understand factors determining the effectiveness of trichomes against herbivores.

412           Regarding associational effects in morphological traits, Wise *et al.* (2009) reported  
413 that resistance to gall flies did not depend on the frequency of the erect-stemmed and candy-  
414 cane phenotypes of *Solidago altissima* in 1.5-m<sup>3</sup> cages. Here, we also found limited evidence  
415 of associational effects between white butterflies and the trichome phenotypes of *A. halleri*.  
416 Given that adult white butterflies can move among plant patches by flying, we speculate that  
417 associational effects are unlikely to cause resistance to oviposition by the butterflies at our 1-  
418 m patch scale (Table 4). Although this study did not detect associational effects, our previous  
419 study found that the magnitude of herbivory on hairy plants depended on the proportion of  
420 glabrous plants within 1-m diameter patches in another population where the flightless leaf  
421 beetle *Phaedon brassicae* was the predominant herbivore on *A. halleri* (Table 4; Sato *et al.*,  
422 2014). Taken together, our data suggest that herbivore mobility affects the occurrence of  
423 associational effects between herbivory and trichome phenotype of *A. halleri* (Table 4).

424           As for another ecological function, plant trichomes are known to interfere with the  
425 foraging behaviour of predators (e.g. Kauffman & Kennedy, 1989; reviewed by Dalin *et al.*,  
426 2008). We observed a parasitoid, *Aphidius* sp., in the experimental plots, but the number of  
427 parasitized aphids was not fewer on hairy plants. Thus this did not support such interfering

428 effects of trichomes on foraging of predators. However, it is still possible that some  
429 confounding interactions among herbivores have hindered associational effects. Firstly, size  
430 reduction of glabrous plants by two leaf chewers (*Pieris rapae* and *Athalia infumata*) may  
431 account for the slightly higher number of *Phyllotreta striolata* on hairy plants in the common  
432 garden. Secondly, the aphid colony contained a specialist, *Lipaphis erysimi*, and a generalist,  
433 *Myzus persicae* (Rout & Senapati, 1968; Le Guigo *et al.*, 2012); therefore, it is possible that  
434 anti-herbivore resistance or its associational effects could have been detected if the two  
435 species had been distinctly evaluated.

436           Although the result that abundance of *Pieris rapae* larvae were reduced on hairy  
437 plants are congruent with the pattern of leaf damage in a natural population where *Pieris napi*  
438 were dominant, one caveat is that *P. rapae* does not utilize *A. halleri* in natural populations  
439 (Ohsaki & Sato, 1999). Ohsaki and Sato (1999) showed that larvae of *P. rapae* were able to  
440 grow on *A. halleri*, but adults did not oviposit on *A. halleri* when they had access to other  
441 cruciferous plants, such as *Brassica* and *Raphanus* species. Thus, our results of the common  
442 garden experiment should be interpreted carefully about to what extent they are comparable  
443 to patterns of leaf damage in a population where a congener, *P. napi*, is the dominant  
444 herbivore.

445           In conclusion, although this study does not provide any conclusive evidence of  
446 associational effects of anti-herbivore resistance, we did find that trichomes can act as a  
447 resistance trait against certain herbivores in *A. halleri*. However, we should note that  
448 conditions under which associational effects can be detected sometimes depend on spatial  
449 scales (Janz *et al.*, 2005; Hambäck *et al.*, 2009). For example, Janz *et al.* (2005) illustrated  
450 that the oviposition preference of a polyphagous butterfly, *Polygonia c-album*, did not depend  
451 on the frequency of *Urtica dioica* and *Salix caprea* within a patch, but such frequency-

452 dependent oviposition was found among plant patches. In our study, it remains possible that  
453 associational effects against flying herbivores (e.g. adults of *Pieris* species) can be detected at  
454 a spatial scale greater than 1 m<sup>2</sup>. Thus, in future studies, it would be valuable to examine the  
455 effects of spatial scales in order to understand when associational effects occur in anti-  
456 herbivore resistance.

457

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465

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584 **Table 1.** Effects of the trichome phenotype and frequency condition on the cumulative number of herbivores and plant performance (the  
585 number of flowers or leaves) in the experiment. Likelihood ratio tests were performed using generalised linear models (GLMMs). Early-  
586 season herbivores (aphids) and late-season herbivores (*Pieris rapae*, *Athalia infumata*, and *Phyllotreta striolata*) were separately analysed.  
587 Plant performance was also separately analysed according to the analyses of herbivore abundance. Main effects were tested first and then  
588 an interaction term was analysed. The bold and underlined values indicate < 5% significance and marginal (5%–10%) significance,  
589 respectively.

Fixed effects	#Wingless aphids			#Winged aphids*			#Mummified aphids*			#Flowers			#Matured fruits**			#Intact leaves		
	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>
Trichome	1	0.7	0.39	1	0.4	0.52	<u>1</u>	<u>3.6</u>	<u>0.06</u>	<b>1</b>	<b>3.9</b>	<b>0.05</b>	1	0.2	0.65	1	0.17	0.68
Frequency	1	0.7	0.39	1	2.5	0.12	1	0.001	0.98	<b>1</b>	<b>4.7</b>	<b>0.03</b>	1	0.01	0.93	1	2.1	0.15
Tri. × Freq.	<b>1</b>	<b>5.7</b>	<b>0.02</b>	1	2.8	0.10	<u>1</u>	<u>3.7</u>	<u>0.05</u>	1	3.5	0.06	1	0.04	0.84	<b>1</b>	<b>10.7</b>	<b>0.001</b>
<i>Residuals</i>	48	860	---	48	73.6	---	48	80.2	---	48	84.9	---	48	32.4	---	48	242.4	---

\*No. of wingless aphids was included as an offset term; \*\*No. of flowers was included as an offset term.

Fixed effects	# <i>P. rapae</i> eggs			# <i>P. rapae</i> larvae			# <i>A. infumata</i> larvae			# <i>P. striolata</i> adults			#Damaged leaves***		
	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>
Trichome	<b>1</b>	<b>5.4</b>	<b>0.02</b>	<u>1</u>	<u>3.3</u>	<u>0.07</u>	1	0.6	0.42	<u>1</u>	<u>3.2</u>	<u>0.07</u>	1	2.3	0.13
Frequency	<u>1</u>	<u>3.0</u>	<u>0.09</u>	1	0.03	0.87	1	0.2	0.62	1	0.2	0.65	1	0.03	0.87
Tri. × Freq.	1	0.01	0.93	1	0.5	0.47	1	0.002	0.97	1	0.5	0.50	<b>1</b>	<b>138.7</b>	<b>&lt; 0.001</b>
<i>Residuals</i>	48	54.8	---	48	98.4	---	48	67.8	---	48	56.1	---	48	791.4	---

\*\*\*No. of intact leaves was included as an offset term.

590 **Table 2.** Insect herbivores counted in the study sites (a) and effects of the trichome phenotype, proportion of glabrous plants in a patch, and  
591 total number of *Arabidopsis halleri* plants in a patch on leaf damage (arcsine-transformed proportion of the leaf area loss) (b) at the  
592 Ojigahata site and (c) at the Minoh site. Likelihood ratio tests were performed using linear mixed models. Main effects were tested first and  
593 then an interaction term was analysed. The bold and underlined values indicate < 5% significance and marginal (5%–10%) significance,  
594 respectively. The patch ID was incorporated as a random effect in these analyses.

(a) Insect herbivores

Species	2011 Autumn		2012 Spring		2012 Autumn		2013 Spring	
	Ojigahata	Minoh	Ojigahata	Minoh	Ojigahata	Minoh	Ojigahata	Minoh
<i>Pieris napi</i> adults*	8	0	8	0	22(9)	0	8(3)	0
<i>Phyllotreta striolata</i> adults	0	0	15	3	2	0	33	0
<i>Athalia infumata</i> larvae	0	0	1	7	0	0	0	0

\*No. of eggs and larvae are shown within parentheses.

(b) Ojigahata

Fixed effects	23 September 2011			27 May 2012			15 September 2012			26 May 2013		
	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>
Trichome	<b>1</b>	<b>20.8</b>	<b>&lt; 0.001</b>	<b>1</b>	<b>7.3</b>	<b>&lt; 0.01</b>	<b>1</b>	<b>4.1</b>	<b>0.04</b>	<b>1</b>	<b>36.1</b>	<b>&lt; 0.001</b>
Proportion of glabrous plants	<b>1</b>	<b>5.9</b>	<b>0.02</b>	1	0.4	0.56	1	0.1	0.76	1	0.9	0.34
Total number of plants	1	0.48	0.49	1	0.05	0.83	<b>1</b>	<b>5.0</b>	<b>0.03</b>	1	2.1	0.15
Tri. × Prop. of glabrous plants	1	0.50	0.48	1	0.01	0.91	<b>1</b>	<b>4.8</b>	<b>0.03</b>	<b>1</b>	<b>4.5</b>	<b>0.03</b>
<i>Residuals</i>	1016	-844.4	---	505	-437.3	---	383	-310.9	---	441	-393.1	---

(c) Minoh

Fixed effects	1 October 2011			3 June 2012			22 September 2012			1 June 2013		
	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>	<i>df</i>	Deviance	<i>P</i>

Trichome	<u>1</u>	<u>3.7</u>	<u>0.06</u>	1	0.01	0.93	<b>1</b>	<b>37.2</b>	<b>&lt; 0.001</b>	<b>1</b>	<b>13.6</b>	<b>&lt; 0.001</b>
Proportion of glabrous plants	1	1.5	0.22	1	0.01	0.93	1	0.8	0.37	<b>1</b>	<b>5.0</b>	<b>0.025</b>
Total number of plants	1	1.0	0.31	<u>1</u>	<u>3.8</u>	<u>0.05</u>	<b>1</b>	<b>11.1</b>	<b>0.001</b>	<b>1</b>	<b>7.3</b>	<b>&lt; 0.01</b>
Tri. × Prop. of glabrous plants	1	1.3	0.26	1	2.2	0.13	1	0.3	0.57	<u>1</u>	<u>3.6</u>	<u>0.06</u>
<i>Residuals</i>	691	-687	---	471	-371.9	---	561	-553.1	---	571	-409.6	---

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605 **Table 3.** Effects of the proportion of glabrous plants in a patch and the total number of *Arabidopsis halleri* plants in a patch on leaf damage  
606 (arcsine-transformed proportion of the leaf area loss) (a) at the Ojigahata site and (b) at the Minoh site. Standardized coefficients and their  
607 standard error (SE) in linear mixed models explaining the leaf damage are shown for the two factors, in which bold values indicate < 5%  
608 significant deviation of coefficients from zero (Wald tests). The patch ID was incorporated as a random effect in these analyses. The  
609 number of plant patches examined is shown within the parentheses beside the survey dates, and the number of hairy or glabrous plants  
610 examined is presented within the parentheses below each survey date. The observational evidence of herbivore fauna was described in  
611 Table 2.

(a) Ojigahata

Fixed effects	23 September 2011 (80)		27 May 2012 (72)		15 September 2012 (90)		26 May 2013 (70)	
	Hairy (498)	Glabrous (523)	Hairy (180)	Glabrous (330)	Hairy (178)	Glabrous (210)	Hairy (156)	Glabrous (290)
Proportion of glabrous plants	-0.07 ± 0.06	<b>-0.12 ± 0.06</b>	0.08 ± 0.08	0.01 ± 0.06	-0.12 ± 0.07	0.10 ± 0.09	0.09 ± 0.08	-0.08 ± 0.07
Total number of plants	-0.02 ± 0.08	-0.02 ± 0.07	0.07 ± 0.08	0.002 ± 0.066	<b>-0.16 ± 0.07</b>	-0.15 ± 0.10	<b>-0.23 ± 0.08</b>	-0.02 ± 0.08

(b) Minoh

Fixed effects	1 October 2011 (67)		3 June 2012 (66)		22 September 2012 (85)		1 June 2013 (70)	
	Hairy (340)	Glabrous (356)	Hairy (186)	Glabrous (290)	Hairy (249)	Glabrous (317)	Hairy (197)	Glabrous (379)
Proportion of glabrous plants	-0.04 ± 0.09	<b>-0.23 ± 0.07</b>	-0.07 ± 0.10	0.07 ± 0.08	-0.03 ± 0.09	-0.08 ± 0.07	<b>0.35 ± 0.08</b>	0.03 ± 0.07
Total number of plants	-0.06 ± 0.11	-0.12 ± 0.09	<b>-0.19 ± 0.10</b>	-0.11 ± 0.08	<b>-0.31 ± 0.13</b>	<b>-0.26 ± 0.09</b>	-0.08 ± 0.09	<b>-0.27 ± 0.10</b>

612 **Table 4.** Patterns of anti-herbivore resistance and associational effects between different herbivores and hairy and glabrous plants of  
613 *Arabidopsis halleri*. Feeding habit, dispersal mode, and body length of these herbivores (less than the maximum size throughout their life  
614 cycle) are also listed. Circles and cross marks indicate that associational effects were supported and not supported, respectively.  
615 Associational effects cannot be discussed regarding herbivores in which trichomes do not serve as a resistance trait (indicated by ‘NA’).

Herbivore	Species	Feeding habit	Dispersal	Body length	Resistance effect of trichomes	Associational effects between trichome phenotypes	References
Leaf beetle	<i>Phaedon brassicae</i>	Chewer	Walking	< 1.0 cm	○	○	Sato <i>et al.</i> , 2014
White butterflies	<i>Pieris rapae</i> , <i>Pieris napi</i>	Chewer	Flying (adult)	< 4.0 cm	○	×	This study
Cabbage sawfly	<i>Athalia infumata</i>	Chewer	Flying (adult)	< 2.0 cm	○	×	This study
Flea beetle	<i>Phyllotreta striolata</i>	Chewer	Jumping	< 0.3 cm	×	NA	This study
Aphids	<i>Myzus persicae</i> , <i>Lipaphis erysimi</i>	Sucker	Walking + Flying	< 0.3 cm	×	NA	This study

616 **Figure legends**

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618 **Figure 1.** A schematic diagram showing the experimental design of the experiment in which  
619 the relative frequency of hairy and glabrous plants was manipulated. The hairy-abundant (H  
620 > G) plot and glabrous-abundant plot (H < G) consisted of 84% and 16% hairy plants,  
621 respectively. Twenty-five plants were planted in a grid, and three replications were set for  
622 each plot type. In the left two panels, a filled or open square indicates a hairy (H) or glabrous  
623 (G) plant, respectively. Analysed plants (nine individuals in the centre of each plot) were  
624 sorted by rosette size, and the number after the 'H' or 'G' indicates the ascending order of  
625 sorting. The rest of the hairy or glabrous plants were placed on the edge of the plots with  
626 randomization of plant size. Plants were maintained at intervals of 20 cm.

627 **Figure 2.** The cumulative number (mean  $\pm$  SE) of herbivorous insects (a–c, f–i) and the  
628 number of flowers (d) and leaves (e, j) of the hairy (H; coloured) and glabrous (G; white)  
629 plants in the hairy-abundant (H > G) and glabrous-abundant (H < G) plots. Data are  
630 separately analysed for the early- and late-season surveys to distinguish the effects of  
631 herbivory between the two periods (see also text and Figs. S1, S2).

632 **Figure 3.** Leaf damage (proportion of the leaf area loss: mean  $\pm$  SE) of hairy (H; coloured)  
633 and glabrous (G; white) plants (a) at the Ojigahata site and (b) at the Minoh site. Data are not  
634 transformed in the figure. Detailed survey dates and observational evidence of herbivore  
635 fauna are provided in Table 2.

636 **Figure 4.** Leaf damage (proportion of the leaf area loss) on hairy (coloured circles) or  
637 glabrous (white circles) plants in relation to the increasing proportion of neighbouring  
638 glabrous plants (a) at the Ojigahata site and (b) at the Minoh site. Circles and vertical bars  
639 respectively indicate the mean and SE of leaf damage on individual plants within a 1-m  
640 diameter patch. Note that many points do not have error bars because all plants within a patch  
641 received the same damage score. Trend lines (dashed lines) were added on the basis of single  
642 regressions when the slope coefficients were significant. Darker circles indicate a larger  
643 number of plants in a patch. Data are not transformed in the figure. Detailed survey dates are  
644 described in Table 2 and 3.

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646 **Figure legends for supporting information**

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649 **Figure S1.** Temporal patterns in the number of each herbivore on the hairy (coloured) and  
650 glabrous (white) plants in the hairy-abundant (left) and glabrous-abundant (right) plots  
651 throughout the study. Mean  $\pm$  SE values are shown.

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654 **Figure S2.** Temporal patterns in the performance of hairy (coloured) and glabrous (white)  
655 plants in the hairy-abundant (left) and glabrous-abundant (right) plots throughout the study.  
656 Mean  $\pm$  SE values are shown. Flowers were counted as the total number of pedicels per plant,  
657 including both fruited and non-fruited flowers.

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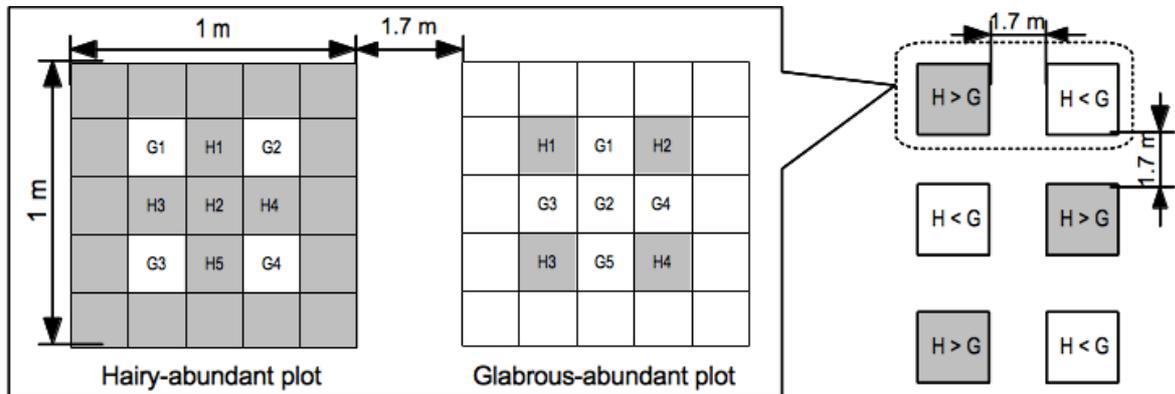
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667 **Figure 1**



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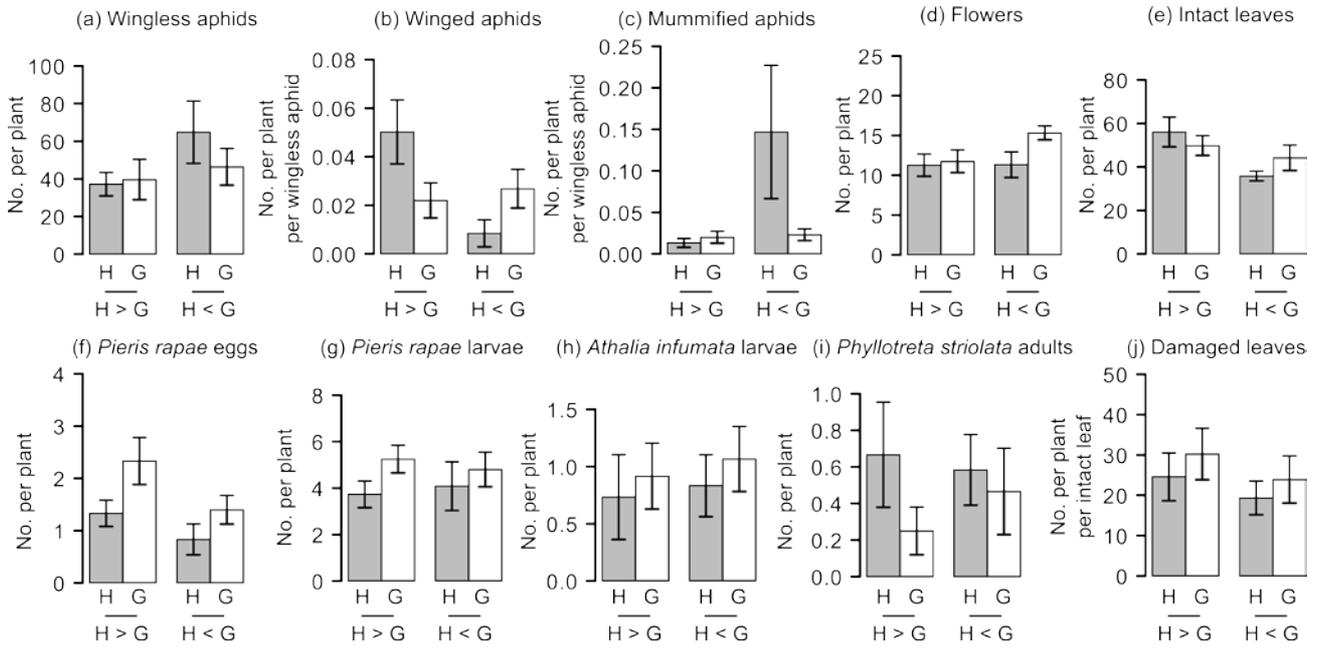
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682 **Figure 2**



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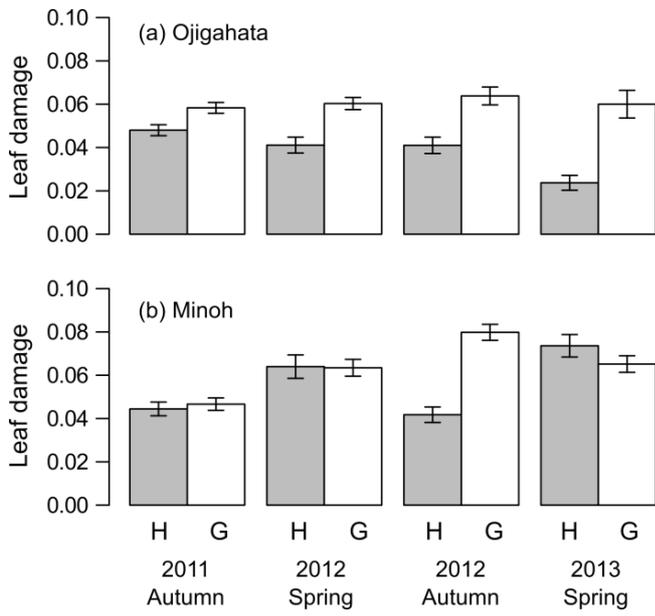
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694 **Figure 3**



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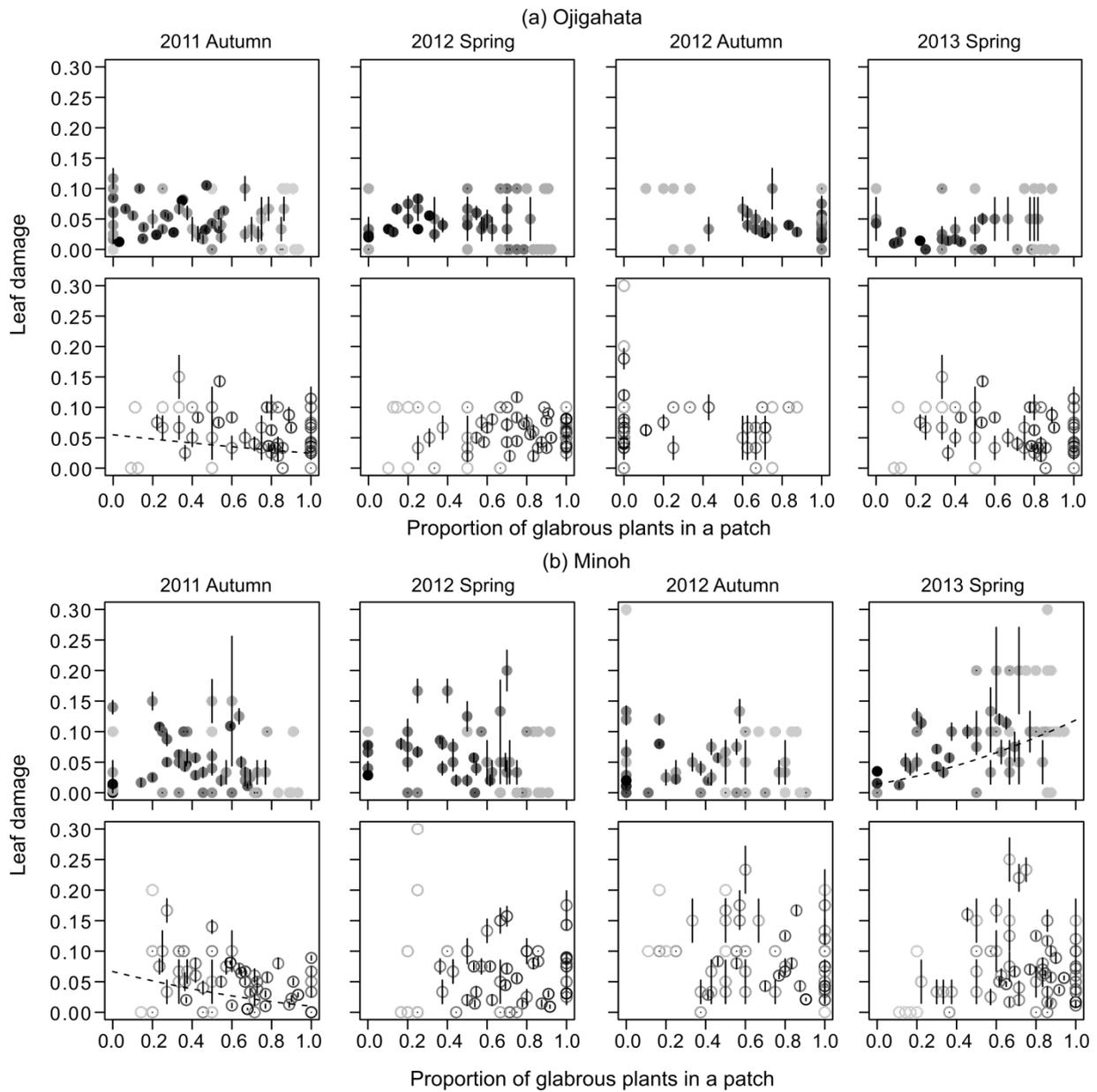
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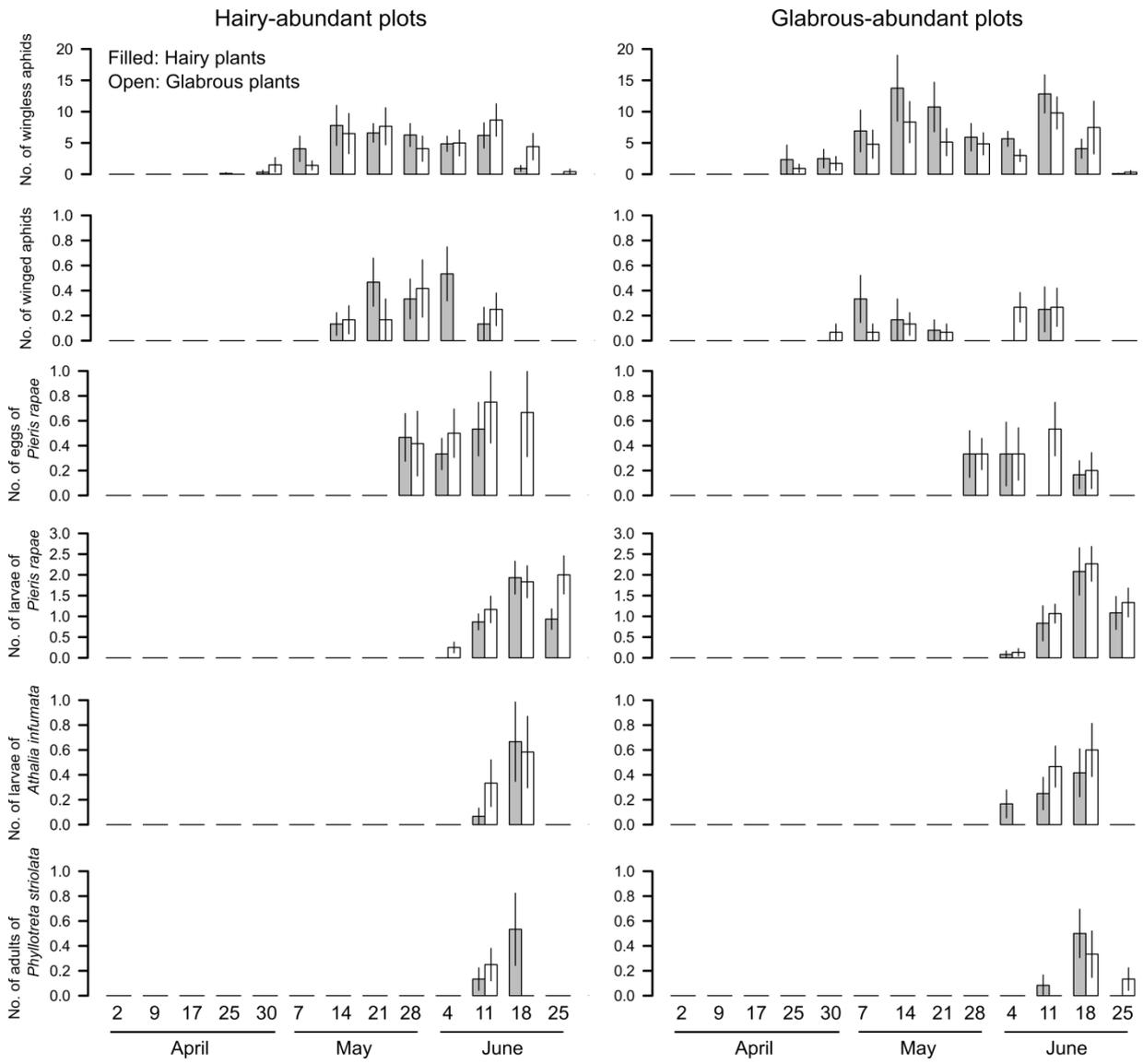
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711 **Figure S1**



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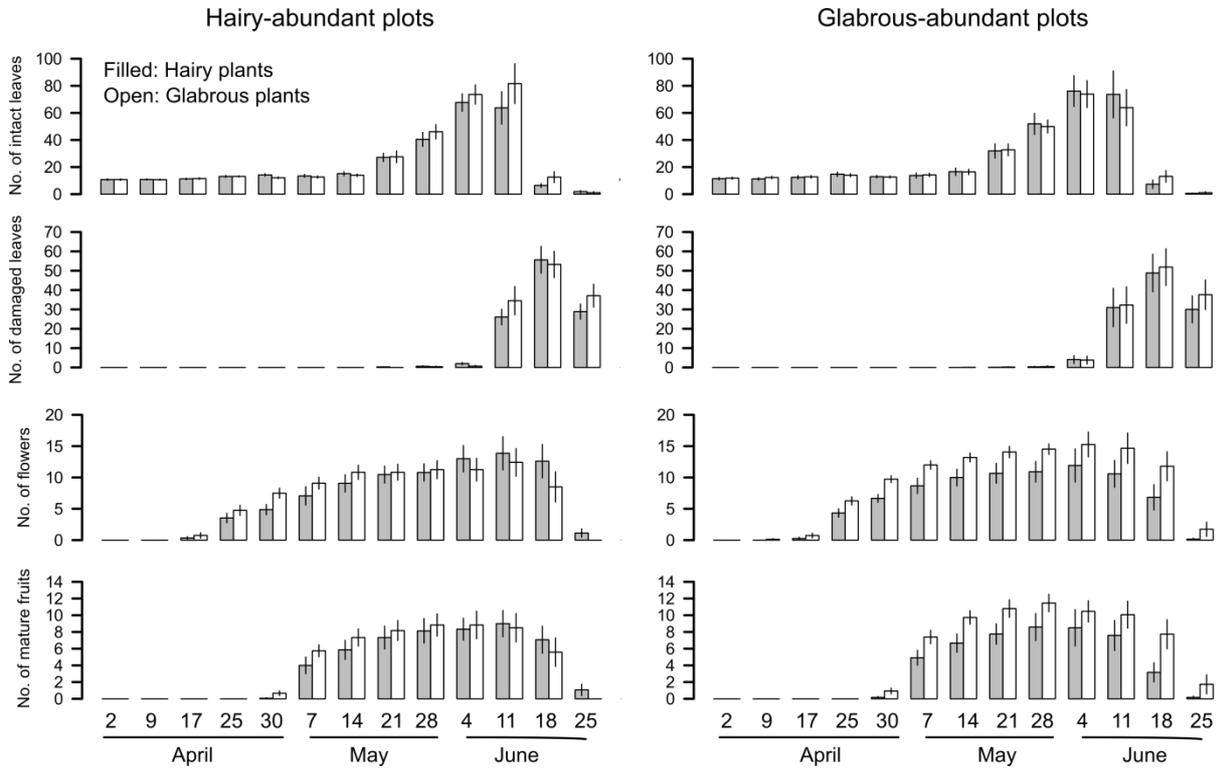
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717 **Figure S2**



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