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1 **Associational effects against a leaf beetle mediate a minority advantage in**  
2 **defense and growth between hairy and glabrous plants**

3

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14

15 Running title: Rarity advantage in plant defense and growth under associational effects

16

17 The main text consists of 6741 words (excluding references, figures, and tables) including

18 Abstract (241 words), Introduction (1006 words), Materials and Methods (3099 words),

19 Results (946 words), Discussion (1338 words), and Acknowledgements (94 words). The

20 entire manuscript consists of the main text with 54 References, 3 figures (without colors), 2

21 tables, and an online appendix including 2 supplemental figures and 3 supplemental tables.

22

23

24

25 **Abstract**

26 Based on the accumulation of evidence, the risk of herbivory depends not only on the traits of  
27 a plant but also on those of neighboring plants. Despite the potential importance of  
28 frequency-dependent interactions in the evolutionary stability of anti-herbivore defense, we  
29 know little about such associational effects between defended and undefended plants within a  
30 species. In this study, we determined whether the intraspecific associational effects against the  
31 oligophagous leaf beetle, *Phaedon brassicae*, caused a minority advantage in defense and  
32 growth between trichome-producing (hairy) and trichomeless (glabrous) plants of *Arabidopsis*  
33 *halleri* subsp. *gemmifera*. We experimentally demonstrated that the magnitude of herbivory  
34 and the number of adult beetles on hairy plants decreased when hairy plants were a minority,  
35 whereas the leaf damage and the beetle abundance did not differ between hairy and glabrous  
36 plants when glabrous plants were a minority. By contrast, the larvae of *P. brassicae* occurred  
37 less when hairy plants were a majority. We also found a reciprocal minority advantage in the  
38 biomass production for both hairy and glabrous plants. Additionally, the adults tended to  
39 attack glabrous leaves more rapidly than hairy ones, particularly when the beetles were  
40 starved or experienced glabrous diets. Furthermore, in the absence of herbivory, the growth of  
41 hairy plants tended to be slower than glabrous plants, which indicated a cost for the  
42 production of trichomes. Our study suggests that associational effects are a mechanism for the  
43 maintenance of trichome dimorphism by contributing to negative frequency-dependent  
44 growth. [241 words]

45

46 **Keywords:** Anti-herbivore defense, *Arabidopsis halleri* subsp. *gemmifera*, Associational  
47 effects, Frequency dependence, Minority advantage, Polymorphism

48

## 49 **Introduction**

50           As sessile organisms, plants are exposed to threats of resource loss by herbivory. To  
51 mitigate the negative effects of losses from herbivory, many plant species develop defensive  
52 traits (e.g., toxins and spines) against herbivores. Based on resource allocation theory of plant  
53 defense, the assumption has long been that the magnitude of herbivory on an individual plant  
54 depends on the investment of that plant in defensive traits (Rhoades 1979; Simms and Fritz  
55 1992; Stamp 2003). However, the accumulation of evidence now indicates that herbivory risk  
56 depends not only on the traits of an individual plant but also on those of neighboring plants  
57 (Agrawal et al. 2006; Barbosa et al. 2009). Some researchers have noted the crucial role of  
58 such 'associational effects' in determining the coexistence of multiple plant types because the  
59 associational effects may mediate the local frequency or the density dependence of herbivory  
60 (Underwood et al. 2014; Kim and Underwood 2015). Although several terminologies have  
61 been proposed for associational effects (reviewed by Barbosa et al. 2009; Rautio et al. 2012;  
62 Underwood et al. 2014), we followed Underwood et al.'s (2014) definition which is  
63 "Associational effects occur when consumer effects on individuals of one resource organism  
64 type, at a given density of that type, are a function of the neighborhood composition of other  
65 resource types at particular spatial scales."

66           Although associational effects are investigated as a type of interspecific interaction  
67 (e.g., Root 1973; White and Whitham 2000; Callaway et al. 2005), the effects can also be  
68 applied to anti-herbivore defense polymorphism within a single species (Tuomi et al. 1999;  
69 Rautio et al. 2012). Based on evolutionary game theory, several authors discuss how defended  
70 and undefended plants coexist under associational interactions (Sabelis and de Jong 1988;  
71 Till-Bottraud and Gouyon 1992; Tuomi et al. 1999). One possible explanation is negative  
72 frequency-dependent selection in which associational effects may allow multiple plant types

73 to persist within a population when herbivory decreases plant fitness and rare types can  
74 escape from herbivory (Tuomi et al. 1999). As a type of associational effect, Bergvall et al.  
75 (2006) introduced the term ‘neighbor contrast defense and susceptibility’ to denote,  
76 respectively, the minority advantage or disadvantage in herbivory on a given plant type.  
77 Additionally, these preceding studies suggest that herbivore perception on contrasting diets is  
78 a pivotal mechanism for that type of associational effect (Bergvall et al. 2006; Rautio et al.  
79 2012). However, empirically, little is known about whether and how associational effects  
80 mediate frequency dependence in defense and growth for defended and undefended plants  
81 within a species (Wise et al. 2009).

82         From previous research on anti-herbivore defense, defensive traits often incur a cost  
83 on the growth and/or reproduction of a plant (Elle et al. 1999; Koricheva 2002; Züst et al.  
84 2011), and optimal defense theory predicts a single defense strategy balanced between  
85 defense and growth (Simms and Fritz 1992; Stamp 2003). In contrast to this prediction,  
86 natural plant populations exhibit genetic polymorphisms in both chemical (Hughes 1991;  
87 Windsor et al. 2005) and physical defenses (Kivimäki et al. 2007; Wise et al. 2009). Generally,  
88 in prey-predator interactions, (1) some fraction of prey abandon defensive traits to avoid the  
89 cost, and (2) predation risks for defended and undefended prey sometimes depends on the  
90 composition of the neighborhood prey (Brower et al. 1970; Speed and Ruxton 2005;  
91 Svenningsen and Holen 2007). At least theoretically, a stable dimorphism of defended and  
92 undefended prey can evolve under predator-mediated interactions (Svenningsen and Holen  
93 2007). Thus, associational effects may provide insights into why anti-herbivore defense  
94 polymorphism is maintained under plant-herbivore interactions.

95         Trichomes (plant epidermal hairs) are a physical defense trait with distinct  
96 phenotypes that are governed by a few primary genes (Kivimäki et al. 2007; Grebe 2012).

97 Plant trichomes are a mechanical barrier against feeding by adult and larval insects and  
98 against ovipositing by adults (e.g., Zvereva et al. 1998; Chu et al. 2001; Sletvold et al. 2010;  
99 Yamawo et al. 2012). Trichomes also function in the tolerance to abiotic stresses (Wagner et  
100 al. 2004). Moreover, trichomes impose a cost on plant growth and/or reproduction (Sletvold et  
101 al. 2010; Kawagoe et al. 2011; Züst et al. 2011). To test intraspecific associational effects and  
102 their frequency dependence, we focused on trichome production as a representative trait of  
103 plant defense that exhibited genetic polymorphism.

104 *Arabidopsis halleri* (L.) O’Kane & Al-Shehbaz subsp. *gemmifera* (Matsum.)  
105 O’Kane & Al-Shehbaz [Brassicaceae/Cruciferae: referred to as *A. halleri*, hereafter] possesses  
106 a genetic dimorphism of trichome-producing (hairy) and trichomeless (glabrous) morphs  
107 (Kawagoe et al. 2011). For this subspecies, Kawagoe et al. (2011) determined the  
108 reproductive cost for the production of trichomes. In an evaluation of the anti-herbivore  
109 resistance conferred by trichomes, Sato et al. (2014) reports that an oligophagous beetle  
110 *Phaedon brassicae* Baly [Coleoptera: Chrysomelidae] preferred to feed on glabrous leaves  
111 and avoided hairy leaves only when the latter leaf type was rare. However, Sato et al. (2014)  
112 used leaf-discs in the feeding experiment, and whether associational effects can be detected at  
113 the whole-plant level is unknown.

114 The purpose of this study was to test whether the associational effects resulted in a  
115 minority advantage in the defense and the growth for the two plant morphs. We  
116 experimentally tested intraspecific associational effects in anti-herbivore defense against a  
117 leaf beetle on *A. halleri* in which leaf damage, herbivore abundance, and plant biomass were  
118 evaluated under contrasting neighborhood conditions of hairy and glabrous plants. We also  
119 performed two additional experiments. First, to examine a possible cause of the associational  
120 effects, we observed the biting behaviors of the leaf beetles on hairy and glabrous leaves

121 because biting is a useful measure of host preference in *Phaedon* species (Kühnle and Müller  
122 2012). In this additional experiment, we preconditioned adult beetles on the two leaf types  
123 with the aim to simulate beetles with experience feeding on hairy-abundant and  
124 glabrous-abundant plants. Second, hairy and glabrous plants were cultivated without  
125 herbivory to ascertain the growth and/or reproductive cost of trichome production. Because  
126 the cost of trichome production can differ depending on plant ontogeny in *Arabidopsis* (Züst  
127 et al. 2011), we measured the growth and reproduction at multiple points in time.

128

## 129 **Materials & Methods**

130

### 131 *Study system*

132 *Arabidopsis halleri* is a self-incompatible perennial herb that is distributed across  
133 Japan and the Russian Far East. In the lowlands of western Japan, plants begin flowering in  
134 late March or early April. After flowering, plants develop leaves and roots on the primary and  
135 axillary meristems of the flowering stems, and these new rosettes establish as clonal offspring  
136 by rooting into the ground. The presence/absence of trichomes is associated with the allelic  
137 status of a trichome-related gene, *GLI*, but not with its flanking regions or other genes  
138 (Kawagoe et al. 2011). Hairy plants develop nonglandular trichomes on the surfaces of leaves  
139 and stems, whereas glabrous plants have no trichomes except for on the leaf margins. From a  
140 natural population that was previously studied (located in central Japan, 35°06' N, 134°56' E,  
141 ca. 200 m in altitude; Kawagoe et al. 2011; Sato et al. 2014), the seeds of *A. halleri* were  
142 collected. The glucosinolate profiles were not associated with the trichome phenotype in the  
143 young leaves collected from our study population (Sato et al. 2014).

144 *Phaedon brassicae* is a pest of cruciferous vegetables (Wang et al. 2007a). Adults of  
145 *P. brassicae* are flightless and access host plants exclusively by walking. The larvae are far

146 less mobile than the adults (Ôtake and Funaki 1958), and therefore, the host plant on which a  
147 larva feeds is determined by adult choice. This species of leaf beetle typically reaches the  
148 adult stage within 3 weeks after hatching, and adults survive for approximately 2 months  
149 under laboratory conditions across various ranges of temperature and photoperiod (Wang et al.  
150 2007a, b). The adults and the last-instar larvae are ca. 4-8 mm in body length. We used a  
151 laboratory-reared population that was previously established and maintained the population at  
152 a temperature of 20°C and a photoperiod of 12:12 h L:D (Sato et al. 2014). The sex of the  
153 beetles was determined based on the body size (males, 0.5 mm smaller in abdomen height and  
154 width than females). To equalize the likelihood of oviposition, we used the sex ratio of 50:50  
155 within each treatment in all of the experiments described below.

156

#### 157 *Greenhouse test of minority advantage in defense and growth*

158 We conducted a greenhouse experiment that used two contrasting neighborhoods of  
159 hairy and glabrous plants to test for the intraspecific associational effects (hairy  
160 plants:glabrous plants = 3:1 and 1:3 individuals; referred to hereafter, as hairy-abundant and  
161 glabrous-abundant conditions, respectively). These two contrasting neighborhood conditions  
162 were adopted because the feeding responses of *P. brassicae* did not differ between the  
163 glabrous-abundant condition and the condition with even proportions of the two morphs (i.e.,  
164 hairy leaves:glabrous leaves = 2:2; Sato et al. 2014).

165 The mature fruits were harvested from 14 maternal plants (seven hairy and seven  
166 glabrous plants) in early July 2013 at our study site; the maternal plants were separated by at  
167 least 5 m to minimize the likelihood of multiple sampling of a single clone. Hundreds of seeds  
168 from each maternal plant were separately sown on Petri dishes (diameter 9 cm, depth 1.5 cm;  
169 Kord-Valmark Co., Ontario, Canada) filled with quartz sand. The seeds were germinated  
170 under 12:12 h L:D conditions with the respective air temperatures of 25°C and 15°C; in these



171 conditions, > 80% of the seeds germinated successfully. We transplanted three seedlings that  
172 each had 2-4 leaves into a plastic pot (diameter 9.0 cm, depth 8.0 cm, Y-pot; SAKATA SEED  
173 CO., Yokohama, Japan) filled with seedling-raising compost (Cat. No. 776-274-01; TAKII &  
174 CO., Ltd., Kyoto, Japan). The compost contained nutrients (N:P:K = 320:210:300 mg/L,  
175 respectively). The plants were grown under 12:12 h L:D with a constant air temperature of  
176 20°C. Water was supplied every 3-4 days during the experiment. The two smallest plants were  
177 harvested from each pot after three months of cultivation to avoid competition within the pots.  
178 The photosynthetically active radiation ranged from 40 to 60  $\mu\text{mol}/\text{m}^2/\text{s}$  on the surface of the  
179 pots (LI-190 Quantum Sensor; LI-COR, Lincoln, NE, USA) for which the red:far-red ratio  
180 was 2.1–2.4.

181           The experiment began 3.5 months after the seedling transplantations: 120 plants  
182 were transferred to 30 nylon nets (23 cm diameter by 33 cm height, supported by a 30-cm  
183 long wood pole, with 75% light-transmittance), with four individuals per net. Fifteen  
184 replicates were established for each neighborhood condition (4 individuals  $\times$  2 neighborhood  
185 conditions  $\times$  15 replicates = 120 plants in total). The pots were adjoined to allow leaf beetle  
186 movement between plants without the necessity of first climbing down to the ground. The  
187 four individual plants within each net were arbitrarily chosen from different maternal plants to  
188 minimize the frequency of bias of the maternal family within a replicate. The smallest plants  
189 (length of largest leaf < 80 mm) were not selected to control for the effects of initial size on  
190 the magnitude of herbivory. The adult beetles were allowed to feed on the *A. halleri*  
191 individuals under the two neighborhood conditions, with two males and two females  
192 randomly chosen from the laboratory-reared population released per net after being starved  
193 for a day.

194           We scored the damage level to each leaf as 0 (intact), 1 (ca. 0% < area loss  $\leq$  25%),

195 2 (25% < loss ≤ 50%), 3 (50% < loss ≤ 75%), 4 (75% < loss < 100%), or 5 (100% loss or only  
196 a petiole remained) at 1, 2, 3, and 4 weeks after the release of the beetles. The damage levels  
197 were evaluated separately for mature and young leaves because these leaf types differed in the  
198 density of trichomes (mean ± SE = 2.3 ± 0.5 and 16.0 ± 1.4 trichomes/0.25 cm<sup>2</sup> for mature  
199 and young leaves, respectively; Wilcoxon signed rank tests,  $V = 0$ ,  $n = 9$  plants,  $P < 0.01$ ) and  
200 in the leaf thickness (specific leaf area, mean ± SE = 252 ± 18 and 380 ± 39 cm<sup>2</sup>/g for mature  
201 and young leaves, respectively;  $V = 55$ ,  $n = 16$  plants,  $P < 0.01$ ). For the growth stages, the  
202 radial leaves developed first at the core of the rosette toward the vertical axis and then  
203 expanded outward toward the horizontal axis. Thus, we used the angle of the leaf petiole as an  
204 indicator of leaf age: the mature leaves were classified as leaves with a petiole angle < 45°,  
205 whereas the young leaves were those with angles ≥ 45°. The larval and adult leaf beetles on  
206 each plant were counted every 3-4 days. Because the eggs of *P. brassicae* are embedded in  
207 plant tissue by adults, it was difficult to distinguish oviposition in plants from injuries; thus,  
208 the newly hatched larvae were retrieved after each count to evaluate oviposition by adult  
209 females in this early period of the greenhouse experiment. For the larvae, we also recorded  
210 the leaf type on which the larvae were found (i.e., young or mature leaf) to evaluate which  
211 type of leaf was chosen for oviposition by the females. To analyze both the intensity and the  
212 duration of the cumulative effects of herbivory, all measurements were repeated until a month  
213 after the release of adult beetles.

214           To examine the effects of trichome phenotype and neighborhood condition on plant  
215 growth, we measured the plant biomass (including the aboveground vegetative organs and the  
216 primary root) of the experimental plants at the conclusion. All plants were harvested 2 months  
217 after the release of the adult beetles. The samples were oven-dried overnight (50°C,  
218 DRK633DB; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). In this later period of the greenhouse

219 experiment, to include the effects of larval feeding on plant growth, we did not remove the  
220 larvae after the last measurement of leaf damage that was referred to above. The dried plants  
221 were weighed to a precision of  $10^{-4}$  g using an electronic analytical scale (GR-60; A&D  
222 Company, Ltd., Tokyo, Japan). We recorded the weight of a dead plant as zero (1 of the 120  
223 plants). The flower production was not evaluated because the plants did not produce flowers  
224 at this stage. No plants and only one beetle died during the first month after the release of the  
225 beetles.

226           Additionally, in the greenhouse experiment, we determined whether the trichome  
227 density of hairy plants was altered by wounding. We cut 50% of the leaf area from 16 hairy  
228 plants that were not used in the aforementioned experiment. A month after the damage, we  
229 counted the trichomes on newly expanded leaves under an 8× magnifying glass. However, the  
230 trichome density of the hairy plants did not increase (intact plant, mean  $\pm$  SE =  $87.8 \pm 14.2$   
231 trichomes/cm<sup>2</sup>,  $n = 8$ ; damaged plant,  $81.5 \pm 10.6$  trichomes/cm<sup>2</sup>,  $n = 8$ ; Mann-Whitney  $U$ -test,  
232  $U = 34$ ,  $P = 1$ ).

233

#### 234 *Feeding preference of adult beetles on hairy and glabrous leaves*

235           We observed the feeding preference of adult beetles on hairy and glabrous leaves as  
236 a complement to the greenhouse experiment. In this observation, we also tested whether  
237 short-term experience with abundant hairy and glabrous diets could enhance/inhibit the host  
238 preference by beetles. Young leaves (1.5-2.0 cm in length) were used that were randomly  
239 harvested from plants grown under the identical conditions described above. Forty-five male  
240 and female adults of *P. brassicae* were randomly selected from the laboratory-reared  
241 population. We placed thirty beetles in each of three transparent plastic cases (diameter 8.0  
242 cm, depth 4.5 cm), and these beetles were preconditioned with hairy or glabrous leaves or

243 were starved. Ten young leaves of either hairy or glabrous plants were supplied to the  
244 pre-hairy and pre-glabrous conditions, respectively. After the preconditioning, each beetle fed  
245 on either a hairy or glabrous leaf within a Petri dish (diameter 6 cm, depth 1.5 cm;  
246 Kord-Valmark Co., Ontario, Canada) with a moistened filter paper (diameter 5.5 cm; Toyo  
247 Roshi Kaisha, Ltd., Tokyo, Japan). We recorded the feeding marks on the leaves every thirty  
248 minutes for six hours after the start of the observations. The presence/absence of feeding  
249 marks was then checked a day after the beginning of the observations. Twenty-four male and  
250 female beetles randomly chosen from each preconditioning treatment were used per  
251 observation (8 beetles  $\times$  2 leaf types  $\times$  3 preconditions). This procedure was repeated three  
252 times (i.e., 144 beetles were examined in total). The observations were conducted under the  
253 identical conditions used to cultivate the experimental plants, as described above.

254

#### 255 *Performance of hairy and glabrous plants without herbivory*

256 We cultivated hairy and glabrous plants without herbivory to compare the growth  
257 and reproductive potentials. The mature fruits were collected from 14 maternal plants (seven  
258 hairy and seven glabrous plants) from our study population in early July 2011. The maternal  
259 plants were separated from each other by at least 5 m. We prepared 5 to 11 plants from each  
260 maternal family in an indoor space without herbivory. Fifty seeds from each maternal family  
261 were sown on a Petri dish (diameter 9 cm, depth 1.5 cm) filled with moistened quartz sand on  
262 August 15, 2011, to germinate at room temperature (ca. 80% of the seeds germinated). Three  
263 seedlings from the identical family were transplanted to the identical pot on November 25,  
264 2011. The plastic pots (diameter 10.5 cm, depth 9 cm) were filled with mixed soil  
265 (pumice:leaf mold:peat moss = 1:1:1). All seedlings had 2 to 4 leaves when transplanted.  
266 These plants were grown inside a transparent plastic case (75 cm  $\times$  45 cm with a depth of 18

267 cm; 28 pots per case) for four months with natural sunlight (10–12 h in day length) at room  
268 temperature (average 10–30°C per day). The locations of the cases were rotated monthly.  
269 Liquid fertilizer (N:P:K ratio = 6:10:5, Hyponex; Hyponex, Osaka, Japan) was diluted  
270 1000-fold and supplied monthly. The photosynthetically active radiation was 800-1100 and  
271 100-300  $\mu\text{mol}/\text{m}^2/\text{s}$  on sunny and cloudy days, respectively. The red:far-red ratio ranged from  
272 1.0 to 1.3 (LI-190 Quantum Sensor; LI-COR, Lincoln, NE, USA).

273         The largest plant of the three in each pot was identified with a wooden pin 1 month  
274 after the transplant, and the marked plants were measured. We recorded the length of the  
275 largest leaf and the numbers of leaves and flowers to assess plant growth and reproduction.  
276 The number of leaves and the leaf lengths were recorded ca. 1, 4, and 6 months after the  
277 transplant (corresponding to December 30, 2011, March 2, 2012, and May 3, 2012,  
278 respectively). Of the three plants in each pot, the smallest two (i.e., the unmarked plants) were  
279 removed 4 months after the transplant to avoid competition within the pots. The flowers were  
280 counted ca. 6 and 7 months after the transplant (corresponding to May 3, and May 26, 2012,  
281 respectively). The fruits were not evaluated because the plants did not produce any fruits  
282 because of self-incompatibility. All plants except for two individuals survived to the end of  
283 the study. The trichome phenotype was identified 6 months after the transplants ( $n_{\text{hairy}} = 68$   
284 and  $n_{\text{glabrous}} = 42$ ) because hairs on the stem identified the phenotype more easily. To follow  
285 the individual plants without destructive sampling, we estimated plant biomass using plant  
286 allometry based on measurements at ca. 4 months after transplant for this growth condition.  
287 We used the length of the largest leaf (cm)  $\times$   $\ln$ [no. of leaves] to estimate  $\ln$ [biomass (mg)]  
288 because this metric explained a large proportion of the variation in biomass (linear regression,  
289  $Y = 0.13X + 2.48$ ,  $R^2 = 0.80$ ; see Figure S1 in Supporting Information).

290

291 *Statistical analyses*

292 We used cumulative link mixed models (Agresti 2002) to analyze the effects of the  
293 trichome phenotype, the neighborhood condition, and the initial plant size on the leaf damage  
294 score. The cumulative model is used to fit ordinal response data (Agresti 2002), and therefore,  
295 the model was applied to the ordinal response of the leaf damage score. We treated the  
296 trichome phenotype, neighborhood condition, and initial plant size as fixed effects.  
297 Additionally, we included an interaction between the trichome phenotype and the  
298 neighborhood condition in the model as a fixed effect to test whether the trichome phenotype  
299 differentially influenced leaf damage depending on the neighborhood conditions of the two  
300 plant types. The length of the largest radial leaf represented the initial plant size, which was  
301 an approximation of the radius of the rosette. We included two clustering variables as random  
302 effects. First, the maternal plant ID was incorporated to consider potential heritable variation  
303 among the maternal families. Second, we considered the individual plant ID among repeated  
304 measures as another random effect. The significance of the fixed effects was analyzed using  
305 likelihood ratio tests in which we first compared the models with or without an interaction  
306 term and then tested each fixed effect using the models without interaction terms. The  
307 coefficients of fixed effects were also examined when performing the likelihood ratio tests.  
308 These approaches of cumulative model analyses were performed separately for the young and  
309 the mature leaves and for the pooled data on the two leaf types to examine whether the  
310 resulting herbivory patterns were specific to the leaf types. Although the damage scores of  
311 individual leaves were used for the statistical analyses, we calculated the proportion of leaf  
312 area loss of an entire plant by averaging the midpoints of the corresponding area loss of the  
313 damage score of the leaves (i.e., damage scores of 0, 1, 2, 3, 4, and 5 = 0.0, 0.125, 0.375,  
314 0.625, 0.875, and 1.0, respectively).

315           We used generalized linear mixed models to analyze the numbers of adult and larval  
316 beetles and linear mixed models (LMMs) to analyze plant biomass. We selected a Poisson  
317 error structure for the generalized LMMs to analyze the count response (i.e., the numbers of  
318 beetles and larvae). According to these cumulative model analyses, likelihood ratio tests were  
319 used to analyze the trichome phenotype, neighborhood condition, interaction between the  
320 trichome phenotype and the neighborhood condition, and initial plant size as fixed effects.  
321 The cumulative number of beetles was analyzed as the response variable to reflect both the  
322 intensity and the duration of herbivory on the plants (Ruppel 1983). For the data on larvae, we  
323 separately analyzed the numbers of larvae on the young, mature and all leaves on an  
324 individual plant. The maternal plant ID was incorporated as a random effect in these analyses.  
325 The biomass data were  $\ln(x + 0.1)$ -transformed to improve normality. Additionally, for the  
326 data of the greenhouse experiment, we compared each pair of the two trichome phenotypes  
327 and the neighborhood conditions using pairwise likelihood ratio tests. The sample size of the  
328 minority phenotypes was only fifteen in our greenhouse experiment; and therefore, the  
329 *p*-values are provided without any correction because of the low statistical power (see also  
330 Nakagawa 2004 for an explanation of multiple comparisons).

331           The time until the initial bite was also analyzed using the cumulative model. The  
332 response, i.e., time until the initial bite, was an ordinal variable, and therefore, it was  
333 converted to scores of 1-14. The scores of 1-12 corresponded to every < 30-minute time  
334 interval up to 6 h, and the scores of 13 and 14 indicated the presence and absence of feeding  
335 after a day, respectively. The trichome phenotype, precondition, and interaction between these  
336 two factors were analyzed as explanatory variables using likelihood ratio tests. We  
337 incorporated the experiment ID as a random effect, but the estimated variance was small (<  
338  $10^{-8}$ ), and several models did not converge. Thus, the random effect was not included in the

339 cumulative model used to explain the timing of bite initiation.

340           For the data from the indoor cultivation, we used LMMs for the estimated biomass  
341 (4 and 6 months after the transplant) and generalized LMMs with a Poisson error structure for  
342 the number of flowers (6 and 7 months after the transplant). The estimated biomass was  
343 ln-transformed to improve normality. The trichome phenotype and maternal plant ID were  
344 analyzed as fixed and random effects, respectively. To incorporate potential size biases in the  
345 initial state, we considered the initial biomass (estimated 1 month after the transplant) as a  
346 covariate for the analysis of the estimated biomass. For the number of flowers, to distinguish  
347 the direct effects of the trichome phenotype on flower production from those mediated by a  
348 growth difference, we also analyzed models with or without a fixed effect of the estimated  
349 biomass 4 months after the transplant. We tested the significance of each fixed effect using  
350 likelihood ratio tests.

351           All statistical analyses were performed using the R statistical software package  
352 version 3.0.0 (R Core Team 2013). We used the clmm function (in the ordinal package;  
353 Christensen 2013) for the cumulative link mixed models, the glmer function (in the lme4  
354 package; Bates et al. 2014) for the generalized or ordinal LMMs, and the vglm function (in  
355 the VGAM package; Yee 2013) for the cumulative models with no random effects.

356

## 357 **Results**

358

### 359 *Minority advantage in defense and growth for hairy and glabrous plants*

360           The magnitude of leaf damage on hairy plants was lower than that on glabrous  
361 plants under the glabrous-abundant condition ( $LR-\chi^2_1 = 14.5$ ,  $P < 0.001$ ), whereas the leaf  
362 damage was not significantly different between hairy and glabrous plants under the



363 hairy-abundant condition ( $\text{LR-}\chi^2_1 = 0.6, P = 0.44$ ; Fig. 1a). At the end of the experiment, the  
364 less abundant type of plant accumulated a larger biomass than that of the abundant type in the  
365 identical neighborhood condition (hairy-abundant,  $\text{LR-}\chi^2_1 = 5.97, P < 0.05$ ; glabrous-abundant,  
366  $\text{LR-}\chi^2_1 = 3.89, P < 0.05$ ) or in the identical type in the other neighborhood condition (hairy  
367 plants,  $\text{LR-}\chi^2_1 = 4.77, P < 0.05$ ; glabrous plants,  $\text{LR-}\chi^2_1 = 7.66, P < 0.01$ ; Fig. 1b). The result  
368 that less abundant hairy plants had less leaf damage compared with the other three treatments  
369 was prominent in young leaves ( $\text{LR-}\chi^2_1 > 9.0, P < 0.01$ ; Table S1 and Fig. 1c). The  
370 differential patterns between the neighborhood conditions were confirmed by a significant  
371 trichome  $\times$  neighborhood interaction for the pooled data of damage on young and mature  
372 leaves, damage on young leaves, and plant biomass (Table 1a). The leaf damage on mature  
373 leaves was far less compared with that on young leaves (Fig. 1d) in which no significant  
374 effects were detected for the trichome phenotype or the neighborhood conditions on the  
375 damage (Table 1a). These results for the leaf damage on pooled, young and mature leaf types  
376 were detected across all points in time (after 1-4 weeks after the release of the beetles; Figure  
377 S2). The initial plant size did not differ between the trichome phenotypes or the neighborhood  
378 conditions (generalized LMM,  $P > 0.2$  for the trichome phenotype, neighborhood condition,  
379 and the interaction). The details for the coefficients of the intercepts of the cumulative models  
380 are in the supporting information (Table S2).

381           Adults occurred less frequently on hairy plants under the glabrous-abundant  
382 condition ( $\text{LR-}\chi^2_1 = 19.7, P < 0.0001$ ), whereas the cumulative number of adults did not differ  
383 significantly ( $< 1$  beetle, on average) between hairy and glabrous plants under the  
384 hairy-abundant condition ( $\text{LR-}\chi^2_1 = 0.93, P = 0.33$ ; Fig. 1g). The difference in occurrence of  
385 adults on hairy and glabrous plants between the neighborhood conditions was confirmed by a  
386 significant trichome  $\times$  neighborhood interaction (Table 1b). More larvae occurred on hairy

387 and glabrous plants under the glabrous-abundant condition than those on the identical plant  
388 type under the hairy-abundant condition (hairy plants,  $\text{LR-}\chi^2_1 = 4.54$ ,  $P < 0.05$ ; glabrous  
389 plants,  $\text{LR-}\chi^2_1 = 17.2$ ,  $P < 0.0001$ ; Fig. 1h), which was a result that was supported by a  
390 significant effect of the neighborhood condition on the abundance of larvae (Table 1b). The  
391 larvae occurred more frequently on the young leaves of glabrous plants under the  
392 glabrous-abundant condition than those under the hairy-abundant condition ( $\text{LR-}\chi^2_1 > 6.0$ ,  $P <$   
393  $0.05$ ; Table S1 and Fig. 1e), and the larvae occurred less often on hairy leaves than glabrous  
394 leaves under the glabrous-abundant condition (although the difference was not significant,  
395  $\text{LR-}\chi^2_1 = 0.6$ ,  $P = 0.7$ ; Fig. 1e). We observed a larger number of larvae on the mature leaves of  
396 hairy plants under the glabrous-abundant condition than those on the other three treatments  
397 ( $\text{LR-}\chi^2_1 > 9.0$ ,  $P < 0.01$ ; Table S1; Fig. 1f). For the herbivore abundance, no significant effect  
398 of initial plant size was detected (Table 1b).

399

#### 400 *Feeding preference of adult beetles on hairy and glabrous leaves*

401 The glabrous leaves were attacked more rapidly than the hairy ones by adult beetles,  
402 and a larger number of hairy leaves remained intact than that of glabrous ones (Fig. 2). We  
403 also detected a significant effect of the trichome phenotype on the timing of bite initiation of  
404 adult beetles (cumulative link models,  $\text{LR-}\chi^2_1 = 4.13$ ,  $P < 0.05$ ). Neither the main effect of the  
405 precondition nor the interaction between the trichome phenotype and the precondition had a  
406 significant effect on the timing of the bite initiation ( $\text{LR-}\chi^2_2 = 0.48$ ,  $P = 0.79$ ;  $\text{LR-}\chi^2_2 = 3.73$ ,  $P$   
407  $= 0.15$ , respectively). However, the timing of the bite initiation suggested no difference  
408 between hairy and glabrous leaves for the hairy-preconditioned beetles (upper row, Fig. 2). To  
409 further examine whether the hairy or glabrous preconditioning affected the bite initiation, we  
410 performed the identical analysis but excluded the data set of the starved precondition. In this

411 case, the biting initiation of adult beetles was affected by the interaction of the leaf type and  
412 the preconditioning at a marginally significant level ( $\text{LR-}\chi^2_1 = 3.2, P = 0.08$ ). Detailed results  
413 for the coefficients of explanatory variables and intercepts are given in the supporting  
414 information (Table S3).

415

#### 416 *Performance of hairy and glabrous plants without herbivory*

417 The estimated biomass of hairy plants was smaller than that of the glabrous ones 4  
418 and 6 months after the transplants (Table 2; Figs. 3a, b). The estimated biomass was not  
419 significantly affected by the initial biomass ( $P > 0.1$ , both for 4 and 6 months; Table 2). The  
420 hairy plants produced fewer flowers than those of glabrous plants 6 months after the  
421 transplant at a marginally significant level ( $P = 0.07$ ; Table 2 and Fig. 3c), although the hairy  
422 plants did not have significantly fewer flowers 7 months after the transplant ( $P = 0.63$ ; Table  
423 2 and Fig. 3d). Trichome production had no significant effect on flower production when the  
424 initial biomass (i.e., estimated biomass at 4 months after the transplant) was incorporated as a  
425 covariate ( $P > 0.05$ , both for 6 and 7 months; Table 2). The initial biomass was positively  
426 correlated to flower production 6 and 7 months after the transplant, at least at a marginally  
427 significant level (Table 2).

428

429

#### 430 **Discussion**

431 We demonstrated experimentally that hairy plants incurred less herbivory by *P.*  
432 *brassicae* when they were surrounded by glabrous plants. This pattern has been reported as a  
433 type of associational effect; specifically, our example corresponded with the ‘neighbor  
434 contrast defense’ (Bergvall et al. 2006; Rautio et al. 2012) because we observed less herbivory

435 on the defended phenotype (hairy plants, in our case) when surrounded by undefended  
436 individuals. We also found a reciprocal minority advantage in biomass production for the  
437 hairy and glabrous plants, which might serve as a potential mechanism to maintain the  
438 polymorphism through negative frequency-dependent selection.

439

#### 440 *Mechanisms responsible for the pattern of associational effects*

441 As a possible mechanism for these associational effects, Bergvall et al. (2006)  
442 suggest that the contrast of food types perceived by herbivores increases their feeding  
443 preference for the more palatable food. In *Phaedon* species, the host preference of adult  
444 beetles was less for *Brassica* cultivars that produced dense trichomes (Kühnle and Müller  
445 2012). Our observation that adult beetles were inclined to attack glabrous leaves more rapidly  
446 than hairy ones also provided support for the negative influence of trichomes on host  
447 preference in *P. brassicae*. Furthermore, when the weak difference in adult abundance  
448 between the two plant morphs under the hairy-abundant condition was compared, the feeding  
449 preference did not differ considerably between hairy and glabrous leaves when adult *P.*  
450 *brassicae* had been preconditioned with hairy diets. Provided with this circumstantial  
451 evidence for feeding preference determined by diet experience, the behavioral response of  
452 adult beetles indicated that plant damage was likely dependent on the neighborhood  
453 conditions for hairy and glabrous plants. The relevance of this mechanism is also apparent  
454 because *P. brassicae* are flightless and access their hosts exclusively by walking (Ôtake and  
455 Funaki 1958), which forces the beetles to choose plants within a small-scale area. It has been  
456 argued that the spillover of herbivores from undefended to defended plants can alter damage  
457 on defended neighbors (spillover effects; White and Whitham 2000); however, in our study,  
458 spillover was not likely because the adult beetles actively avoided hairy plants.

459 Trichome density is often high on young leaves (e.g., Chu et al. 2001; Yamawo et al.  
460 2012), and consequently, leaf age can influence the effectiveness of trichomes as a defense  
461 against herbivory (Chu et al. 2001; Puentes and Ågren 2013). In our study, the pattern that  
462 less abundant hairy plants suffered less herbivory was the most prominent for the young  
463 leaves because the young leaves of hairy plants produced denser trichomes than those of  
464 mature leaves. Therefore, trichomes might play an important role in defending the younger  
465 leaves that contribute to the future growth of *A. halleri*. Although the pattern of larval  
466 abundance between hairy and glabrous plants was not clear, the larvae tended to occur more  
467 often on the young leaves of glabrous plants under the glabrous-abundant condition. Because  
468 the larvae of *P. brassicae* rarely move between plants, the abundance of newly hatched larvae  
469 likely reflected the oviposition patterns of adult females. Thus, the tendency for a larger  
470 number of larvae to occur on young glabrous leaves led to the presumption that the adult *P.*  
471 *brassicae* preferred to oviposit on glabrous leaves.

472 At least at the scale of our experiment, we did not detect significant effects of plant  
473 size on the number of *P. brassicae* for either the larvae or the adults; thus, we hypothesized  
474 that *P. brassicae* responded to the trichome phenotype rather than the resource abundance.  
475 However, it was still possible that density as well as frequency of hairy and glabrous plants  
476 might result in the pattern of leaf damage, because our experiment with two neighborhood  
477 conditions could not determine the relative importance of density- and frequency-dependent  
478 damage on the two plant types (but see Kim and Underwood 2015). We should also note that  
479 when herbivores respond nonadditively to plant density (i.e., group effects; Rautio et al. 2012)  
480 it may lead to similar patterns of damage for an individual plant as those caused by  
481 associational effects (see also Hambäck et al. 2014 for a theoretical consideration). In future  
482 studies, the density and the frequency of the two plant types should be manipulated

483 simultaneously (i.e., response surface design; Damgaard 1998; Inouye 2001) to examine  
484 potential interactions between the associational and the resource concentration/dilution effects  
485 on plant damage (Underwood et al. 2014; Hambäck et al. 2014).

486

#### 487 *Maintenance of polymorphism under associational effects*

488           Classically, a reciprocal minority advantage (or negative frequency-dependent  
489 selection) leads to the coexistence of multiple species/genotypes because a reciprocal  
490 minority advantage can prevent rare types from going extinct (reviewed by Ayala and  
491 Campbell 1974). Our study uncovered a reciprocal minority advantage in plant growth  
492 between the hairy and glabrous morphs, which might be a mechanism that maintains the  
493 dimorphism within populations. The minority advantage could have a number of possible  
494 explanations. First, as assumed in earlier game theories related to associational effects  
495 (Augner et al. 1991; Tuomi et al. 1999), the defended plants might be at a disadvantage in  
496 growth because of the cost of defense. In the indoor cultivation study, we also found that the  
497 growth and reproduction of glabrous plants were slightly but consistently higher than those of  
498 hairy plants in the absence of herbivores. The potential costs of trichomes may even out  
499 defense superiority of hairy plants when the defense is weakened under the hairy-abundant  
500 condition. However, under the glabrous-abundant condition, the escape from herbivory  
501 benefited hairy plants. The associational effects and the costs of defense presumably led to the  
502 reciprocal minority advantage in biomass for the two morphs, although these two factors are  
503 mutually nonexclusive. Second, the larvae became less abundant when hairy plants were a  
504 majority, whereby the less abundant glabrous plants might be protected by the abundant hairy  
505 plants (i.e., associational resistance) and therefore grow faster than hairy ones under the  
506 hairy-abundant condition. To distinguish the associational effects and the plant growth

507 potentials, studies must manipulate the presence/absence of herbivores as well as the  
508 neighborhood condition.

509         Hairy and glabrous plants coexist in a natural population in which *P. brassicae* is  
510 the primary consumer of *A. halleri* (Kawagoe et al. 2011; Sato et al. 2014). In this field site of  
511 our previous study, we found that leaf damage on hairy plants decreased as the frequency of  
512 neighboring glabrous plants within a 1-m patch scale increased (Sato et al. 2014). Although it  
513 remains unknown how important biomass production is in the lifetime fitness of *A. halleri* in  
514 the field, the growth before flowering could be a major component of fitness because the  
515 fecundity of hairy plants depends on their size before flowering (Kawagoe and Kudoh 2010).  
516 In the indoor cultivation described in the present study, the biomass in the growth stage was  
517 also positively correlated with flower production. In addition to sexual reproduction, *A.*  
518 *halleri* produces clonal rosettes after flowering, and this clonal propagation is expected to  
519 depend on biomass at the growth stage. There are no trichomes on the flowers of hairy and  
520 glabrous plants, and if associational effects influence pollinator attraction, it will occur  
521 indirectly through flower production. If plant neighbor affects host-searching abilities and  
522 colonization rates of herbivores between patches in the field, then we must test whether  
523 associational effects result in negative frequency-dependent selection in a natural population.

524

### 525 *Conclusions and implications*

526         Our study suggests that intraspecific associational effects serve as a mechanism for  
527 the maintenance of defense dimorphism by contributing to negative frequency-dependent  
528 growth between defended and undefended plants. Notably, associational effects may cause  
529 frequency-dependent selection not only in plant-herbivore interactions but also in  
530 plant-pollinator and prey-predator interactions (Underwood et al. 2014). For example, the

531 learning behaviors of pollinators can maintain flower color polymorphism via negative  
532 frequency-dependent selection (Gigord et al. 2001). As for plant-pathogen interactions, the  
533 evidence is suggestive for frequency-dependent infection between resistant and susceptible  
534 plants (e.g., Brunet and Mundt 2000; Siemens and Roy 2005). Thus, the findings of this study  
535 position associational anti-herbivore defense in the broad context of mutualistic and  
536 antagonistic interactions involving plants. Future studies should incorporate such intraspecific  
537 associational effects because these effects might explain how genetic polymorphism is  
538 maintained under plant-consumer interactions.

539

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548

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684 **Table 1** Effects of trichome phenotype, neighborhood condition, and initial plant size on traits related to plant damage and growth (a)  
 685 and herbivore abundance (b).

(a) Plant damage and growth

Fixed effects	<i>df</i>	Damage on pooled leaf types*			End-point biomass		
		Coef. ± SE	LR- $\chi^2$	<i>P</i>	Coef. ± SE	LR- $\chi^2$	<i>P</i>
Trichome (Hairy)	1	<b>-0.39 ± 0.11</b>	<b>13.09</b>	<b>&lt; 0.001</b>	-0.03 ± 0.08	0.11	0.74
Neighborhood (H>G)	1	0.12 ± 0.10	1.41	0.23	0.032 ± 0.09	0.03	0.86
Initial leaf length	1	0.002 ± 0.003	0.59	0.44	<b>0.008 ± 0.002</b>	<b>12.9</b>	<b>&lt; 0.001</b>
Tri. × Neigh.	1	<b>0.56 ± 0.20</b>	<b>7.67</b>	<b>&lt; 0.01</b>	<b>-0.55 ± 0.17</b>	<b>9.9</b>	<b>&lt; 0.01</b>

Fixed effects	<i>df</i>	Damage on young leaves*			Damage on matured leaves*		
		Coef. ± SE	LR- $\chi^2$	<i>P</i>	Coef. ± SE	LR- $\chi^2$	<i>P</i>
Trichome (Hairy)	1	<b>-0.52 ± 0.13</b>	<b>15.15</b>	<b>&lt; 10<sup>-4</sup></b>	0.02 ± 0.32	0.004	0.95
Neighborhood (H>G)	1	0.20 ± 0.13	2.27	0.13	-0.58 ± 0.33	3.15	0.08
Initial leaf length	1	0.003 ± 0.003	0.83	0.36	-0.002 ± 0.008	0.05	0.82
Tri. × Neigh.	1	<b>0.86 ± 0.24</b>	<b>11.93</b>	<b>&lt; 0.001</b>	-0.14 ± 0.65	0.04	0.83

(b) Herbivore abundance

Fixed effects	<i>df</i>	Larvae on young leaves			Larvae on matured leaves		
		Coef. ± SE	LR- $\chi^2$	<i>P</i>	Coef. ± SE	LR- $\chi^2$	<i>P</i>
Trichome (Hairy)	1	0.004 ± 0.123	0.0009	0.98	<b>0.53 ± 0.18</b>	<b>9.07</b>	<b>&lt; 0.01</b>

Neighborhood (H>G)	1	<b>-0.24 ± 0.12</b>	<b>4.43</b>	<b>&lt; 0.05</b>	<b>-0.95 ± 0.18</b>	<b>29.9</b>	<b>&lt; 10<sup>-7</sup></b>
Initial leaf length	1	-0.001 ± 0.003	0.16	0.69	-0.001 ± 0.005	0.37	0.85
Tri. × Neigh.	1	0.47 ± 0.24	3.84	0.050	<b>-1.06 ± 0.34</b>	<b>9.31</b>	<b>&lt; 0.01</b>

Fixed effects	<i>df</i>	Adults per plant			Larvae per plant		
		Coef. ± SE	LR- $\chi^2$	<i>P</i>	Coef. ± SE	LR- $\chi^2$	<i>P</i>
Trichome (Hairy)	1	<b>-0.23 ± 0.11</b>	<b>4.00</b>	<b>&lt; 0.05</b>	0.20 ± 0.10	3.72	0.054
Neighborhood (H>G)	1	0.09 ± 0.11	0.59	0.44	<b>-0.46 ± 0.10</b>	<b>22.8</b>	<b>&lt; 10<sup>-5</sup></b>
Initial leaf length	1	0.003 ± 0.003	1.25	0.26	-0.002 ± 0.002	0.41	0.52
Tri. × Neigh.	1	<b>1.05 ± 0.26</b>	<b>18.5</b>	<b>&lt; 10<sup>-4</sup></b>	-0.10 ± 0.19	0.25	0.62

686 **Notes:** Results of mixed models (coefficients and their standard errors) and likelihood ratio (LR- $\chi^2$ ) are shown for each fixed effect with  
687 its degree of freedom (*df*), where bold values indicate < 5% significance. Coefficients of the neighborhood condition were estimated for  
688 the hairy-abundant condition (H>G). We tested the interaction term firstly and then analyzed the main effects of each factor. The initial  
689 plant size was represented by the length of the largest leaf. An asterisk (\*) indicates response variables analyzed by cumulative link  
690 models, whereas no mark indicates those analyzed by generalized or ordinal linear mixed models.

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695 **Table 2** Effects of trichome phenotype on growth and reproduction of *Arabidopsis halleri* subsp. *gemmifera* cultivated under no  
 696 herbivory.

Fixed effects	<i>df</i>	4 months			6 months			7 months					
		Estimated biomass			Estimated biomass			No. of flowers					
		Coef ± SE	LR- $\chi^2$	<i>P</i>	Coef ± SE	LR- $\chi^2$	<i>P</i>	Coef ± SE	LR- $\chi^2$	<i>P</i>			
Trichome (single)	1	<b>-0.36 ± 0.09</b>	<b>16.3</b>	<b>&lt; 0.001</b>	<b>-0.25 ± 0.12</b>	<b>4.2</b>	<b>&lt; 0.05</b>	-0.13 ± 0.07	3.3	0.07	0.02 ± 0.05	0.23	0.63
Trichome (multiple)	1	<b>-0.36 ± 0.09</b>	<b>16.8</b>	<b>&lt; 0.001</b>	<b>-0.26 ± 0.12</b>	<b>4.4</b>	<b>&lt; 0.05</b>	0.12 ± 0.07	2.7	0.10	0.06 ± 0.05	1.2	0.27
Initial biomass*	1	-0.56 ± 0.56	1.01	0.31	-0.87 ± 0.76	1.32	0.25	<b>0.71 ± 0.07</b>	<b>108</b>	<b>&lt; 10<sup>-16</sup></b>	0.09 ± 0.05	3.2	0.07

697 **Notes:** Results of mixed models (coefficients and their standard errors) and likelihood ratio (LR- $\chi^2$ ) are shown for each fixed effect with  
 698 its degree of freedom (*df*). Bold values indicate < 5% significance with likelihood ratio tests. The effect of trichome phenotypes was  
 699 tested with or without the covariate of initial biomass (denoted as single and multiple, respectively). Biomass and flowers were evaluated  
 700 4, 6, and 7 months after transplant, where the biomass was estimated on the basis of plant allometry (see Materials and Method for  
 701 details). Estimated biomass 1 or 4 months after transplant was used as the initial biomass for the analyses of biomass or flowers,  
 702 respectively.

703 **Figure legends**

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705 **Fig. 1** Associational effects in defense and growth of hairy (H; grey) and glabrous (G;  
706 white) plants under hairy-abundant ( $H > G$ ) and glabrous-abundant ( $H < G$ ) conditions.  
707 Shown are leaf damage (proportion of leaf area loss) for pooled data of young and mature  
708 leaves (a), plant biomass (b), damage for young (c) and mature leaves (d), the cumulative  
709 number of larvae on young leaves (e), larvae on mature leaves (f), adult beetles per plant (g),  
710 and larvae per plant (h). Leaf damage was presented as values at four weeks after the  
711 release of beetles. The number of larvae reflected oviposition by female adults because  
712 newly hatched larvae were retrieved after each count. The plant biomass was measured two  
713 months after the release of adult beetles. Different letters indicate  $< 5\%$  significant  
714 differences with pairwise likelihood ratio tests by mixed models that incorporated maternal  
715 plant ID as a random factor. Error bars indicate SEM of the raw data. Sample sizes are  
716 given within parentheses.

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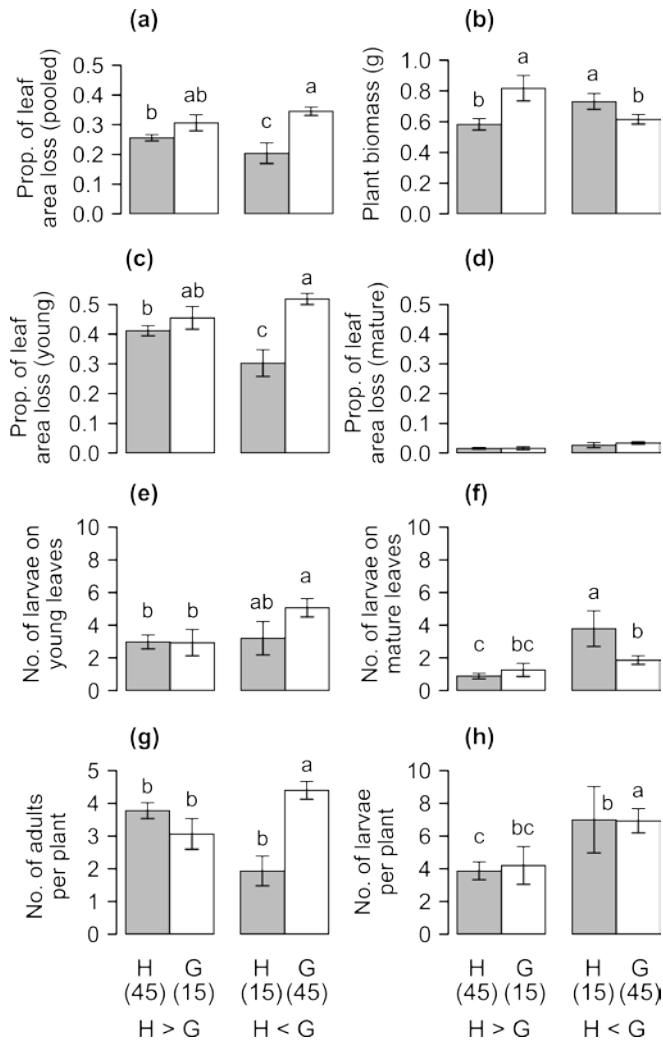
718 **Fig. 2** Time to initial biting by adult *Phaedon brassicae* on hairy (a) and glabrous (b) leaves.  
719 Histograms are shown for overnight preconditioning on hairy (Precon. = Hairy, upper row)  
720 and glabrous leaves (Precon. = Glabrous, middle row) and for beetles starved overnight  
721 (Precon. = None, lower row). Grey and black bars indicate the presence and absence of  
722 feeding marks a day after the release, respectively.

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724 **Fig. 3** Estimated biomass (mg) and the number of flowers of hairy (H; grey) and glabrous  
725 (G; white) plants under no herbivory. Figures are shown for plant growth 4 and 6 months  
726 after the transplant (a and b) and flowering 6 and 7 months after the transplant (c and d).  
727 The biomass was estimated based on plant allometry at ca. 4 months after transplant. Error  
728 bars indicate SEM of the raw data. Data are not transformed in these figures. Sample sizes  
729 are given within parentheses. Asterisks and dots indicate statistical significances (\*\* $P <$   
730 0.001, \*  $P < 0.05$ , •  $P < 0.1$ ).



731 **Figure 1**



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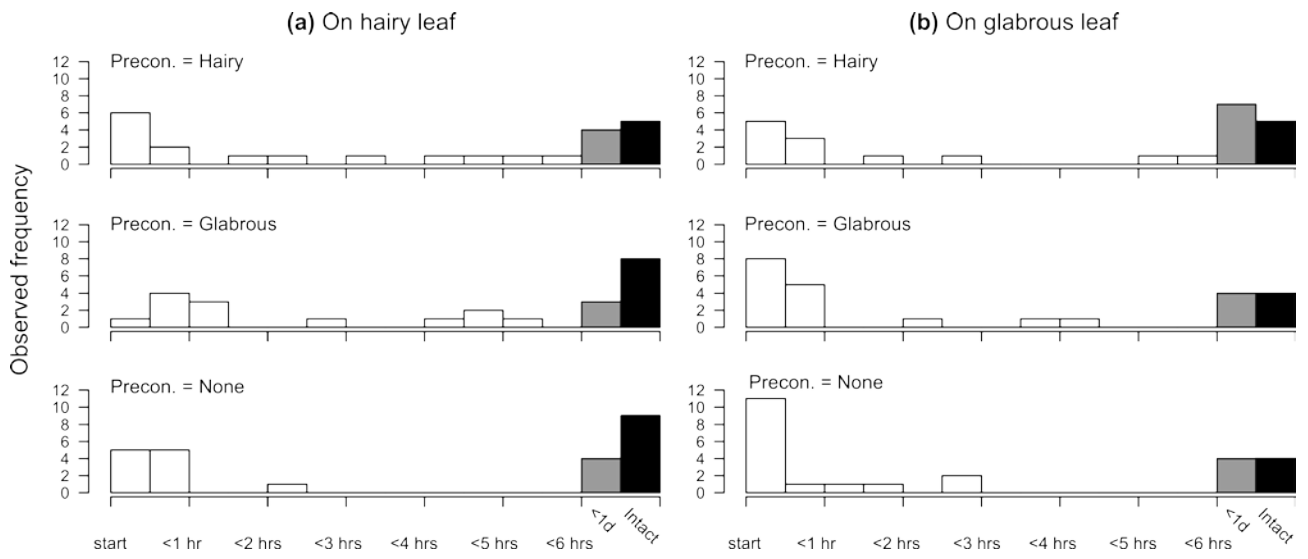
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740 **Figure 2**



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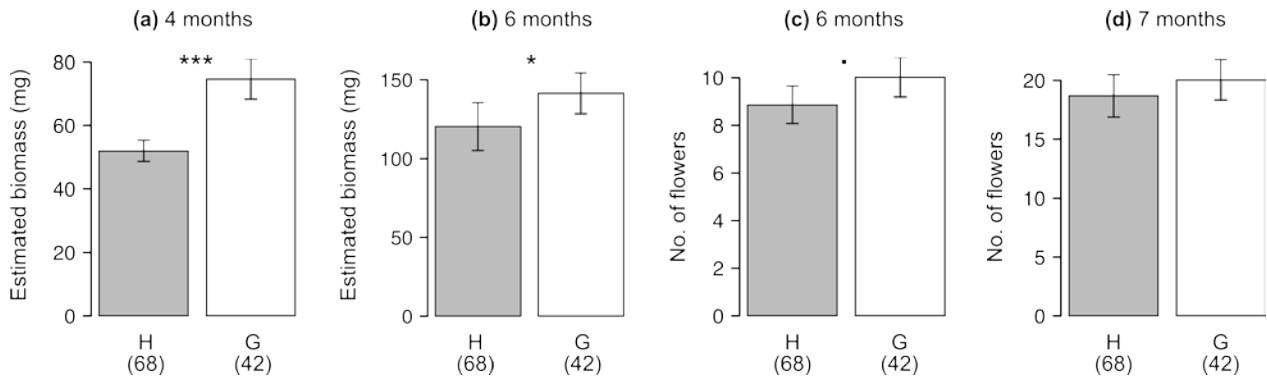
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756 **Figure 3**



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775 **SUPPORTING INFORMATION**

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777 **Table S1** Results of pairwise likelihood ratio tests for the greenhouse experiment data.

778 **Table S2** Intercepts for the leaf damage scores in cumulative link mixed models.

779 **Table S3** Coefficients and intercepts of the time to initial biting by an adult beetle in the  
780 cumulative models.

781 **Figure S1** Biomass (mg) estimation of *Arabidopsis halleri* subsp. *gemmifera* by the number  
782 and length of leaves.

783 **Figure S2** Leaf damage data (proportion of leaf area loss) until four weeks after the release  
784 of leaf beetles.

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799 **Table S1** Results of pairwise likelihood ratio tests for the greenhouse experiment data  
800 including four groups of the trichome phenotypes (H: hairy plants; G: glabrous plants)  
801 plants under two neighborhood conditions (H>G: hairy-abundant condition; H<G:  
802 glabrous-abundant condition).

Response	Group 1		Group 2		LR- $\chi^2$	P
	Tri.	Neigh.	Tri.	Neigh.		
Damage (pooled)	<b>H</b>	<b>H&gt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>7.69</b>	<b>&lt;0.01</b>
	G	H>G	G	H<G	1.53	0.22
	H	H>G	G	H>G	0.60	0.44
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>14.5</b>	<b>&lt;0.001</b>
	<b>H</b>	<b>H&lt;G</b>	<b>G</b>	<b>H&gt;G</b>	<b>7.86</b>	<b>&lt;0.01</b>
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&gt;G</b>	<b>4.86</b>	<b>&lt;0.05</b>
Damage (young)	<b>H</b>	<b>H&gt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>11.7</b>	<b>&lt;0.001</b>
	G	H>G	G	H<G	2.24	0.13
	H	H>G	G	H>G	0.03	0.86
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>20.9</b>	<b>&lt;10<sup>-5</sup></b>
	<b>H</b>	<b>H&lt;G</b>	<b>G</b>	<b>H&gt;G</b>	<b>9.15</b>	<b>&lt;0.01</b>
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&gt;G</b>	<b>4.52</b>	<b>&lt;0.05</b>
Damage (mature)	H	H>G	H	H<G	1.97	0.16
	G	H>G	G	H<G	1.19	0.28
	H	H>G	G	H>G	0.02	0.90
	G	H<G	H	H<G	0.19	0.66
	H	H<G	G	H>G	1.10	0.29
	G	H<G	H	H>G	2.49	0.11
Plant Biomass	<b>H</b>	<b>H&gt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>4.77</b>	<b>&lt;0.05</b>
	<b>G</b>	<b>H&gt;G</b>	<b>G</b>	<b>H&lt;G</b>	<b>7.66</b>	<b>&lt;0.01</b>
	<b>H</b>	<b>H&gt;G</b>	<b>G</b>	<b>H&gt;G</b>	<b>5.98</b>	<b>&lt;0.05</b>
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>3.89</b>	<b>&lt;0.05</b>
	H	H<G	G	H>G	0.37	0.55
	G	H<G	H	H>G	0.13	0.91
Adult beetles	<b>H</b>	<b>H&gt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>14.1</b>	<b>&lt;0.001</b>

	<b>G</b>	<b>H&gt;G</b>	<b>G</b>	<b>H&lt;G</b>	<b>5.42</b>	<b>&lt;0.05</b>
	H	H>G	G	H>G	0.94	0.33
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>19.7</b>	<b>&lt;10<sup>-5</sup></b>
	H	H<G	G	H>G	3.24	0.07
	G	H<G	H	H>G	1.60	0.21
Larvae per plant	<b>H</b>	<b>H&gt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>4.54</b>	<b>&lt;0.05</b>
	<b>G</b>	<b>H&gt;G</b>	<b>G</b>	<b>H&lt;G</b>	<b>17.2</b>	<b>&lt;10<sup>-4</sup></b>
	H	H>G	G	H>G	<0.001	0.98
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>7.67</b>	<b>&lt;0.01</b>
	H	H<G	G	H>G	3.66	0.56
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&gt;G</b>	<b>12.4</b>	<b>&lt;0.001</b>
Larvae on young leaves	H	H>G	H	H<G	1.02	0.31
	<b>G</b>	<b>H&gt;G</b>	<b>G</b>	<b>H&lt;G</b>	<b>14.2</b>	<b>&lt;0.001</b>
	H	H>G	G	H>G	0.62	0.43
	G	H<G	H	H<G	0.10	0.76
	H	H<G	G	H>G	0.04	0.84
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&gt;G</b>	<b>6.21</b>	<b>&lt;0.05</b>
Larvae on mature leaves	<b>H</b>	<b>H&gt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>26.0</b>	<b>&lt;10<sup>-6</sup></b>
	G	H>G	G	H<G	2.25	0.13
	H	H>G	G	H>G	1.49	0.22
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&lt;G</b>	<b>19.1</b>	<b>&lt;10<sup>-4</sup></b>
	<b>H</b>	<b>H&lt;G</b>	<b>G</b>	<b>H&gt;G</b>	<b>9.52</b>	<b>&lt;0.01</b>
	<b>G</b>	<b>H&lt;G</b>	<b>H</b>	<b>H&gt;G</b>	<b>9.22</b>	<b>&lt;0.01</b>

803 **Notes:** Effects of initial plant size and maternal plants were considered as a covariate and  
804 random factor in mixed models, respectively (see text for details). Bold values indicate <5%  
805 significant differences between groups.

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810 **Table S2** Intercepts for the leaf damage scores in cumulative link mixed models.

Intercept	(a) Pooled leaf types			(b) Young leaves			(c) Matured leaves		
	Coefficients	SE	Z	Coefficients	SE	Z	Coefficients	SE	Z
0   1	0.48	0.29	1.68	-0.23	0.35	-0.65	<b>2.76</b>	<b>0.89</b>	<b>3.10</b>
1   2	<b>1.32</b>	<b>0.29</b>	<b>4.58</b>	<b>0.75</b>	<b>0.35</b>	<b>2.16</b>	<b>6.12</b>	<b>0.93</b>	<b>6.59</b>
2   3	<b>1.82</b>	<b>0.29</b>	<b>6.32</b>	<b>1.33</b>	<b>0.35</b>	<b>3.83</b>	NA	NA	NA
3   4	<b>2.16</b>	<b>0.29</b>	<b>7.52</b>	<b>1.72</b>	<b>0.35</b>	<b>4.95</b>	NA	NA	NA
4   5	<b>2.54</b>	<b>0.29</b>	<b>8.83</b>	<b>2.13</b>	<b>0.35</b>	<b>6.12</b>	NA	NA	NA

811 **Notes:** Numbers of intercept column indicate the damage score 0 - 5 that correspond to  
812 intact leaves - complete area loss, respectively. For example, 0|1 indicates a ratio of  
813 probability of being the damage score 0 on that of the score 1. The estimates were  
814 calculated using models including all of the fixed effects. Bold values indicate a deviation  
815 of coefficients from zero significant at  $P < 0.05$  (Wald test). NA indicates no data available.

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829 **Table S3** Coefficients and intercepts of the time to initial biting by an adult beetle (order 1 -  
 830 14) in the cumulative models.

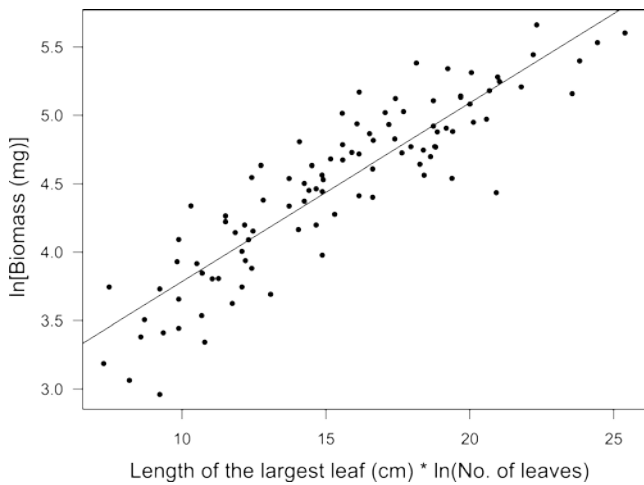
Factors	Coefficient	SE	Z
Trichome (Hairy)	<b>-0.60</b>	<b>0.30</b>	<b>-2.02</b>
Precon. (Hairy)	-0.04	0.36	-0.11
Precon. (None)	0.20	0.36	0.56
Tri. × Precon. (Hairy)	1.18	0.73	1.62
Tri. × Precon. (None)	-0.05	0.73	-0.07
Intercept (1 2)	-0.67	0.38	-1.77
Intercept (2 3)	0.01	0.37	0.03
Intercept (3 4)	0.13	0.37	0.35
Intercept (4 5)	0.22	0.37	0.58
Intercept (5 6)	0.30	0.37	0.82
Intercept (6 7)	0.42	0.37	1.12
Intercept (7 8)	0.45	0.37	1.20
Intercept (8 9)	0.48	0.37	1.28
Intercept (9 10)	0.56	0.37	1.51
Intercept (10 11)	0.65	0.38	1.73
Intercept (11 12)	<b>0.74</b>	<b>0.38</b>	<b>1.96</b>
Intercept (12 13)	<b>0.80</b>	<b>0.38</b>	<b>2.11</b>
Intercept (13 14)	<b>1.64</b>	<b>0.40</b>	<b>4.15</b>

831 **Notes:** Ordered score 1 - 12 correspond to 30-minute intervals after the beginning of  
 832 observation, respectively. Score 13 and 14 indicates the presence and absence of biting  
 833 marks after one day, respectively. For example, 0|1 indicates a ratio of probability of being  
 834 the damage score 0 on that of the score 1. These intercept estimates were calculated using  
 835 models including all of the explanatory variables. Bold values indicate a deviation of  
 836 coefficients from zero significant at  $P < 0.05$  (Wald test).

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839 **Figure S1** Biomass (mg) estimation of *Arabidopsis halleri* subsp. *gemmaifera* by the number  
840 and length of leaves. The biomass, leaf number, and leaf length were evaluated at ca. 4  
841 months after transplant. Solid line represents the prediction by simple linear regression ( $Y =$   
842  $0.13X + 2.48$ ,  $R^2 = 0.80$ ). Details of the growth conditions are described in the main text  
843 (see Materials & Methods).

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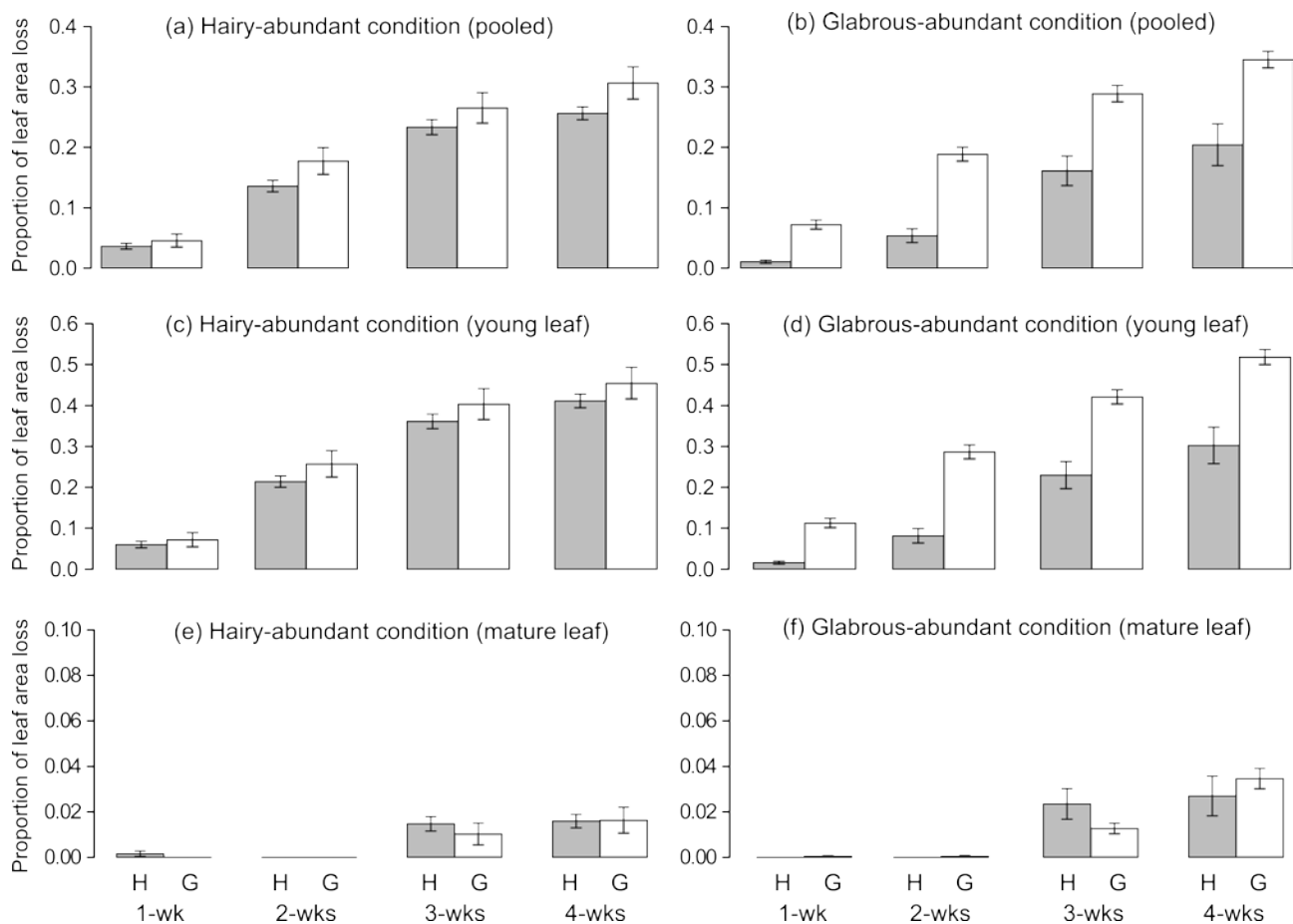
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856 **Figure S2** Leaf damage data (proportion of leaf area loss) until four weeks after the release  
 857 of adult beetles. Data are separately presented for pooled dataset of young and mature  
 858 leaves (a, b), young (c, d), or mature (e, f) leaf type. Mean  $\pm$  SE values of the raw data are  
 859 shown for hairy (H; grey) and glabrous (G; white) plants under the hairy-abundant and  
 860 glabrous-abundant conditions.

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