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1     **Associational effects and the maintenance of polymorphism in plant**  
2             **defense against herbivores: review and evidence**

3  
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5  
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25 **Abstract**

26 Many plant species have evolved defense traits against herbivores. *Associational effects*  
27 (AE) refer to a kind of apparent interaction where the herbivory risk to a focal plant  
28 species depends on the composition of other plant species in a neighborhood. Despite  
29 ample evidence for AE between different plant species, this point of view has rarely  
30 been applied to polymorphism in defense traits within a plant species. The purpose of  
31 this review is to highlight an overlooked role of conspecific AE in maintaining  
32 polymorphism in antiherbivore defense. First, I present a general review of AE between  
33 plant species and its role in the coexistence of plant species. This viewpoint of AE can  
34 be applied to genetic polymorphism within a plant species, as it causes frequency- and  
35 density-dependent herbivory between multiple plant types. Second, I introduce a case  
36 study of conspecific AE in the trichome-producing (hairy) and glabrous plants of  
37 *Arabidopsis halleri* subsp. *gemmifera*. Laboratory and semi-field experiment illustrated  
38 that AE against the brassica leaf beetle *Phaedon brassicae* mediate a minority advantage  
39 in defense and fitness between hairy and glabrous plants. Combined with a statistical  
40 modeling approach, field observation revealed that conspecific AE can maintain the  
41 trichome dimorphism via negative frequency-dependent selection in a plant population.  
42 Finally, I discuss spatial and temporal scales at which AE contribute to shaping genetic  
43 variation in antiherbivore defense in a plant metapopulation. Based on the review and  
44 evidence, I suggest that AE play a key role in the maintenance of genetic variation  
45 within a plant species.

46

47 **Keywords:** Frequency-dependent selection, Genetic variation, Herbivory, Neighbor  
48 effects, Plant defense

49 **1. Background**

50 Many plant species have evolved defensive traits, such as spines and toxins, against  
51 herbivores (Schoonhoven *et al.* 2005). Natural plant populations exhibit genetic  
52 variation in regard to chemical (Hughes 1991; van Dam *et al.* 1999; Chan *et al.* 2010)  
53 and morphological defenses (Kivimäki *et al.* 2007; Wise *et al.* 2009). It is well known  
54 that these defense traits are costly for plant growth and/or reproduction (e.g., Mauricio  
55 1998; Elle *et al.* 1999; Züst *et al.* 2011). The theory of plant defense assumes that the  
56 defense–growth tradeoff favors an optimal balance between the cost and benefit of  
57 defense (Simms 1992), extinguishing variation in defense levels. Thus, it is difficult for  
58 natural selection to maintain genetic variation in antiherbivore defense traits unless a  
59 stabilizing mechanism occurs.

60 Plant defense theory has focused on how individual plants allocate their  
61 resource to defensive traits (e.g., Rhoades 1979; Simms 1992; Stamp 2003). However,  
62 the defensive effects of a particular trait depend not only on a plant’s own trait but also  
63 on the composition of the other plant types in a neighborhood (Agrawal *et al.* 2006;  
64 Barbosa *et al.* 2009; Underwood *et al.* 2014). These phenomena are called *associational*  
65 *effects* (AEs: also known as *neighbor effects*), which occur when “consumer effects on  
66 individuals of one resource organism type, at a given density of that type, are a function  
67 of the neighborhood composition of other resource types at particular spatial scales”  
68 (Underwood *et al.* 2014). An increasing number of studies have shown the importance  
69 of AE in maintaining plant species diversity (e.g., Hay 1986; Callaway *et al.* 2005;  
70 Stastny & Agrawal 2014), but this point of view has rarely been tested in the  
71 evolutionary ecology of antiherbivore defenses.

72 The purpose of the present review is to highlight the overlooked but potentially

73 critical role of conspecific AE in the maintenance of genetic variation in antiherbivore  
74 defense traits. This paper consists of three chapters, in which I first outline the  
75 ecological roles of AE in maintaining plant species diversity. Second, I introduce a case  
76 study on *Arabidopsis* trichomes to expand the concept of AE to polymorphism in  
77 defense within a plant species. Finally, I discuss how AE contributes to intraspecific  
78 variation in plant defense at larger spatial and temporal scales. Based on the review and  
79 evidence, the present paper suggests that AE plays a key role in maintaining not only  
80 species diversity but also genetic variation in antiherbivore defense.

81

#### 82 *1-1. Examples of AE in plant-herbivore interaction*

83 The phenomena of associational resistance or susceptibility have been reported across  
84 agricultural (Tahvanainen & Root 1972; Risch 1981; Le Guigo *et al.* 2012), forest  
85 (White & Whitham 2000; Sholes 2008; Castagneyrol *et al.* 2013), grassland (Agrawal  
86 2004; Courant & Fortin 2010; Hahn & Orrock 2016), semi-arid (González-Teuber &  
87 Gianoli 2007), wetland (Rand 2003; Hughes 2012), and aquatic (Hay 1986; Wahl &  
88 Hay 1995) ecosystems (see Appendix for selected examples). Two major patterns have  
89 been reported for AE between plant species: Associational *resistance* or *susceptibility*  
90 refer, respectively, to a situation where a focal plant species is *less* or *more* damaged in  
91 the presence of another plant species. undefended plants may gain “associational  
92 resistance” when the defended plants are protected from herbivory by nearby plants  
93 (Tahvanainen & Root 1972; Rausher 1981; Hambäck *et al.* 2000). In the polyculture of  
94 the collard green *Brassica oleracea* var. *acephala* (Brassicaceae), Root and colleagues  
95 for the first time developed the concept of “associational resistance” and “resource  
96 concentration,” which refer to the effects of vegetation composition and total density on

97 herbivore populations, respectively (Tahvanainen & Root 1972; Root 1973). Recently, it  
98 was also discovered that neighboring plants do not always reduce herbivory damage to  
99 other plant species. The term “associational susceptibility” was coined to refer to the  
100 situation where defended plants suffer from severe herbivory when they occur nearby  
101 undefended ones (White & Whitham 2000; Rand 2003). Barbosa *et al.* (2009) introduce  
102 many more examples of AE and Rautio *et al.* (2012) outline the terminology regarding  
103 AE, comprising the terms “plant defense guild” (Atsatt & O’Dowd 1976), “shared  
104 doom” (Wahl & Hay 1995; Emerson *et al.* 2012), and “associational refuge” (Hjältén *et*  
105 *al.* 1993; Miller *et al.* 2009; Emerson *et al.* 2012; Hughes 2012).

106         The mechanisms underlying AE are involved in various combinations of plant  
107 traits and herbivore behaviors. In general, herbivores alter their preferences and/or  
108 performance depending on their dietary menu (e.g., Lefcheck *et al.* 2013; Sato & Kudoh  
109 2016a; Wetzel *et al.* 2016). In particular, theory of optimal foraging suggests that  
110 consumers should utilize suboptimal resource as the density of optimal resource  
111 becomes low (e.g., Charnov 1976). This behavioral basis of consumers directly alters  
112 the consumption rate and thus drives apparent competition between optimal and  
113 suboptimal resource (Holt & Kotler 1987). Empirically, mammalian herbivores often  
114 avoid spiny or toxic herbs and thereby lead these defended herbs to confer associational  
115 resistance to undefended plant species at a patch level (e.g., Hjältén *et al.* 1993;  
116 Callaway *et al.* 2005; Courant & Fortin 2010; Miller *et al.* 2009). Visual or chemical  
117 crypsis conferred by neighboring plants results in associational resistance against insect  
118 herbivores (Tahvanainen & Root 1972; Rausher 1981; Hambäck *et al.* 2000). Volatile  
119 organic chemicals from neighboring plants sometimes induce plant resistance traits and  
120 thus drive associational resistance against herbivores (Karban & Maron 2002; Karban

121 2007; Zakir *et al.* 2013). Direct competition sometimes increases plants' investment in  
122 defense traits and may promote associational resistance (Barton & Brower 2006;  
123 González-Teuber & Gianoli 2007). Polyphagous herbivores move from primary to  
124 secondary host species during their dispersal process, resulting in associational  
125 susceptibility on the part of the former to the latter host plant (White & Whitham 2000;  
126 Agrawal 2004). These examples suggest that the outcomes of AE depend on herbivore  
127 behaviors and plant trait changes.

128         While long recognized as apparent competition, the outcomes of AE are more  
129 complex than simple competition between two plant species. AE comprise facilitation,  
130 exploitation, and other kinds of species interaction (Agrawal 2004; Barbosa *et al.* 2009).  
131 In particular, recent reviews pointed out problems with the terminologies and  
132 experimental designs used to test AE (Rautio *et al.* 2012; Underwood *et al.* 2014), as  
133 there has been little consensus regarding the trait of a focal plant type (i.e., defended or  
134 undefended) and manipulations on the neighborhood composition (presence/absence,  
135 relative abundance, or total density of multiple plant types) (see also Appendix). For  
136 example, a number of studies compared the magnitude of herbivory on one of two plant  
137 species between the presence and absence of another species (Hambäck *et al.* 2000;  
138 White & Whitham 2000); however, this setting is unable to determine the likelihood of  
139 the coexistence of two plant species (Underwood *et al.* 2014). Many studies focused on  
140 the absolute amount of herbivory (Tahvanainen & Root 1972; Hambäck *et al.* 2000;  
141 White & Whitham 2000), while others compared the relative amounts of herbivory  
142 between multiple plant types (Bergvall *et al.* 2006; Castagneyrol *et al.* 2013).  
143 Furthermore, some studies focused on the herbivory load to individual plants within a  
144 small spatial scale (referred to as a "patch": Rausher 1981; Hambäck *et al.* 2000;

145 Karban & Maron 2002) while others compared herbivory among the plant patches at  
146 broader spatial scales (Bergvall *et al.* 2006; Castagneyrol *et al.* 2013). Details of the  
147 experimental design and outcomes are listed for 16 publications (see Appendix).

148

149 *1-2. Frequency/density-dependent damage caused by AE*

150 Recently, several authors have begun to discuss how AE enable the coexistence of  
151 multiple plant species. Underwood *et al.* (2014) advocated that the focus should be on  
152 the frequency- or density-dependent damage caused by AE to multiple plant species. If  
153 AE is more likely to protect rare plant species than abundant species, this rarity  
154 advantage (i.e., negative frequency dependence) in defense would prevent rare species  
155 from going extinct due to herbivory, thereby promoting the coexistence of multiple  
156 plant species. In this refined framework, negative or positive frequency-dependent  
157 damage indicates associational resistance or susceptibility for rare plant species,  
158 respectively. Even if multiple plant species can coexist via AE, recovery from low  
159 density (i.e., negative density dependence) is necessary for the long-term persistence of  
160 plant populations. The negative or positive density-dependent damage refers to the  
161 “resource concentration or delusion” hypothesis, initially developed by Root (1973) and  
162 recently reviewed by Underwood *et al.* (2014) and Hambäck *et al.* (2014).

163         Several studies suggest the importance of plant frequency and density in  
164 determining the outcome of AE, although those showing the importance of both the  
165 density and frequency dependence are still limited (Table 1). Kim and Underwood  
166 (2015) manipulated the density and frequency of the tall goldenrod *Solidago altissima*  
167 (Asteraceae) and the Carolina horsenettle *Solanum carolinense* (Solanaceae). As a result,  
168 *S. carolinense* received more damage due to the combined effects of the high

169 conspecific density and intermediate frequency of the two species (Kim & Underwood  
170 2015). Hahn and Orrock (2016) manipulated both the density and frequency of two  
171 related species *Solidago nemoralis* and *S. odora* (Asteraceae) in the field and then  
172 revealed herbivore foraging to be a key mechanism with a behavioral assay. The less  
173 defended species *S. nemoralis* gained associational resistance when it was rare due to  
174 the reduced foraging activity of grasshoppers, with the two *Solidago* species more likely  
175 attacked at a higher density (Hahn & Orrock 2016). In relation to herbivore foraging,  
176 Verschut *et al.* (2016) showed that low-concentration of balsamic vinegar was less  
177 likely to attract the fruit fly *Drosophila melanogaster* at a high frequency of high  
178 concentration diets, while high-concentration diets were more likely to attract flies at a  
179 high frequency of low-concentration diets. Previous studies on the fallow deer *Dama*  
180 *dama* manipulated the tannin concentration as well as the frequencies of two resource  
181 types (Bergvall & Leimar 2005; Bergvall *et al.* 2006). High tannin diets were consumed  
182 less frequently when low tannin diets were abundant, whereas low tannin diets were  
183 consumed more frequently when high tannin diets were abundant (Bergvall & Leimar  
184 2005). Furthermore, if two species co-occur in their natural habitats, field surveys help  
185 us capture the effects of plant density or frequency in the wild population. Russell and  
186 Louda (2005) documented that the native thistle *Cirsium undulatum* (Asteraceae)  
187 received less florivory from the exotic weevil *Rhinocyllus conicus* as the number of  
188 another native thistle (*C. canescens*) increased in close vicinity. This long-term survey  
189 found that an invasive herbivore creates a novel AE due to the overlap of flowering  
190 phenology between two native plant species (Russell & Louda 2004; Russell & Louda  
191 2005).

192           Although density- or frequency-dependent herbivory have been shown

193 experimentally, we still know little about how AE occurs and contributes to the  
194 maintenance of plant species diversity. Thus, it is necessary to link the mechanisms and  
195 patterns of AE in a single study system. There is a common set of empirical approaches  
196 for understanding the mechanisms and consequences of AE from laboratory to the field  
197 study (Fig. 1: see also Appendix for details). The first step is to discover candidate  
198 mechanisms of AE (Fig. 1), including the identification of key plant traits and herbivore  
199 behaviors, and then to address how they cause non-additive herbivory between multiple  
200 plant species. Previous studies have done this by conducting laboratory choice  
201 experiments (e.g., Tahvanainen & Root 1972; White & Whitham 2000; Rand 2003) or  
202 by observing herbivore movements between plants (Rausher 1981; Bergvall *et al.* 2006).  
203 The second step includes experimental tests to determine whether plant damage and/or  
204 fitness depend on the neighborhood composition. This step is adopted by almost all the  
205 studies and is the most important for illustrating the relevance of AE. The third step is  
206 multi-year field survey to corroborate the experimental evidence with field observations  
207 on plant damage and population dynamics across years (Fig. 1). This final step is  
208 executable if the focal plant species co-occur within natural populations (Russell &  
209 Louda 2004; Callaway *et al.* 2005). These comprehensive tests from laboratory  
210 experiment to field observations will reveal the causes and consequences of AE in a  
211 single study system.

212

### 213 *1-3. Plant defense polymorphism via the lens of conspecific AE*

214 While the concept of AE was originally developed in interspecific interactions  
215 (Tahvanainen & Root 1972; Root 1973), it can be applied to genetic variation within the  
216 same plant species. In agricultural ecosystems, polyculture has been carried out not only

217 for different species but also for different cultivars within the same plant species (e.g.,  
218 Cantero & Sanford 1984; Hambäck *et al.* 2009). The emerging framework of  
219 “community genetics” may be considered a kind of conspecific AE (Hughes *et al.* 2008),  
220 as these studies have shown that the polyculture of different genotypes alters herbivore  
221 abundance (e.g., Crutsinger *et al.* 2006; Johnson *et al.* 2006; Parker *et al.* 2010). Studies  
222 on community genetics also revealed that plant genetic diversity altered the strength of  
223 selection on plant genotypes via changes in plant–herbivore interaction (e.g., Johnson *et*  
224 *al.* 2006; Lankau & Kliebenstein 2009; Parker *et al.* 2010), although the way in which  
225 frequency-dependent selection occurs between plant genotypes remains to be explored.

226 Evolutionary game theory provides a logical basis for the roles of AE in  
227 maintaining polymorphism in defense levels (Sabelis & de Jong 1988; Augner *et al.*  
228 1991; Till-Bottraud & Gouyon 1992; Tuomi *et al.* 1999). Theoretical studies analyzed  
229 conditions under which the benefits from AE and the intrinsic cost of defense allow  
230 defended and undefended plants to coexist. In clonal plants, for example, Till-Bottraud  
231 and Gouyon (1992) predicted that herbivore preference and the cost of defense lead to  
232 the evolutionary stable strategy of producing cyanogenic and acyanogenic clones at an  
233 intermediate frequency. Empirically, this hypothesis regarding evolutionary stability can  
234 be tested by asking whether or not rarer plant types have higher fitness compared to  
235 abundant ones under AE (reviewed by Rautio *et al.* 2012). If a rarity fitness advantage  
236 reciprocally occurs among multiple plant genotypes, AE leads to negative  
237 frequency-dependent selection that can prevent rare genotypes from going extinct  
238 (Clarke 1964; Ayala & Campbell 1974). This criterion would allow empirical  
239 researchers to test whether AE can be a mechanism maintaining polymorphism in  
240 antiherbivore defense.

241           Despite its logical basis, only a few studies have focused on conspecific AE  
242 with regard to the maintenance of polymorphism in antiherbivore defense (Table 1).  
243 Wise *et al.* (2009) tested AE in the tall goldenrod *Solidago altissima*, which possesses a  
244 genetically based phenotype of ducking stem (called the “candy-cane” morph). This  
245 candy-cane morph occurs with a rare frequency but across natural populations of *S.*  
246 *altissima* (Wise 2009). Wise *et al.* (2009) conducted a manipulative experiment with  
247 two contrasting frequencies of candy-cane and erect-stem morphs (Table 1). The tall  
248 goldenrod received less damage from the specialist gall fly *Eurosta solidaginis* when  
249 the candy-cane morphs were abundant; however, the damage was frequency  
250 independent, as shown by the lack of a significant morph-by-frequency interaction  
251 (Wise *et al.* 2009). Recently, Garrido *et al.* (2016) tested frequency-dependent selection  
252 between tolerant (i.e., defense mitigating negative effects of herbivory on plant fitness)  
253 and resistant (defense directly reducing herbivore attacks) genotypes in the jimson weed  
254 *Datura stramonium* (Solanaceae), but found a rarity “disadvantage” against the  
255 maintenance of defense polymorphism. Thus, it remains unknown whether conspecific  
256 AE can be a mechanism for the maintenance of defense dimorphism. My collaborators  
257 and I have investigated AE against a leaf beetle in the trichome dimorphism of  
258 *Arabidopsis halleri* subsp. *gemmifera* (Brassicaceae). In the next chapter, I will  
259 introduce an example of conspecific AE and its stabilizing effect on a plant defense  
260 polymorphism.

261

## 262 **2. Evidence**

263 Several species of the genus *Arabidopsis* exhibit a dimorphism of trichome-producing  
264 (hairy) and trichomeless (glabrous) plants. The glabrousness of *Arabidopsis* species is

265 associated with the loss of function of the key gene of trichome development,  
266 *GLABRA1* (*GLI* also known as *GLABROUS1*) (Hauser *et al.* 2001; Kivimäki *et al.*  
267 2007; Kawagoe *et al.* 2011; Bloomer *et al.* 2012). Because *GLI* is a transcriptional  
268 factor gene for the initiation of trichome development, the loss of function of this gene  
269 results in distinct phenotypes of hairy and glabrous plants (Oppenheimer *et al.* 1991;  
270 Hülkamp 2004; Ishida *et al.* 2008). The glabrous phenotype is recessive to the hairy  
271 ones, and these phenotypes are inherited in the Mendelian fashion (Kärkkäinen & Ågren  
272 2002). Laboratory studies and field surveys have shown that hairy plants incurred less  
273 herbivory by insect herbivores compared to glabrous plants (Handley *et al.* 2005;  
274 Sletvold *et al.* 2010; Løe *et al.* 2007; Kivimäki *et al.* 2007), while trichomes impose a  
275 fitness cost on plants (Mauricio 1998; Sletvold *et al.* 2010; Züst *et al.* 2011). Owing to  
276 the visible and discrete phenotypes with the antiherbivore function, *Arabidopsis*  
277 trichomes provide an excellent system to investigate AE against herbivores within a  
278 same-plant species.

279         Likewise, *Arabidopsis halleri* subsp. *gemmifera* (abbreviated hereafter as *A.*  
280 *halleri*) have a dimorphism of hairy and glabrous plants (Fig. 2a). Hairy plants have  
281 non-glandular trichomes on their leaves and stems, whereas glabrous plants produce no  
282 trichomes, except on their stem and leaf margin. This presence/absence of leaf  
283 trichomes is associated with the allelic status of a *GLI* orthologue (Kawagoe *et al.*  
284 2011). Hairy plants showed a lower fecundity and slower growth than glabrous ones did  
285 in the absence of herbivores (Kawagoe *et al.* 2011; Sato & Kudoh 2016b; Sato & Kudoh  
286 2017b), indicating a fitness cost of the trichome production. Further, trichome  
287 production is unlinked with glucosinolate profiles (Sato *et al.* 2014), which may act as a  
288 chemical defense for Brassicaceae (e.g., Züst *et al.* 2012).

289 *Arabidopsis halleri* subsp. *gemmifera* is a self-incompatible perennial herb  
290 distributed across Japan and the Russian Far East. Plants bloom from early April to May  
291 at the lowland of Japan (Kawagoe & Kudoh 2010). Flowers are pollinated by hoverflies  
292 and solitary bees. Seeds are dispersed by gravity. After flowering, plants produce clonal  
293 rosettes on the main and lateral meristems (Sato & Kudoh 2017b), wherein late-spring  
294 herbivory can directly affect the sexual and asexual reproduction. *Arabidopsis halleri*  
295 subsp. *gemmifera* is attacked by multiple species of herbivorous insects including  
296 beetles, butterflies, and sawflies from late-spring to early-summer (Sato & Kudoh 2015;  
297 Sato & Kudoh 2017a). Among natural populations of *A. halleri*, the brassica leaf beetle,  
298 *Phaedon brassicae* (Fig. 2a), occasionally outbreaks and impacts fruit production by  
299 heavily infesting flowering stems (Kawagoe & Kudoh 2010).

300 In this second chapter, I introduce a case study of conspecific AE against the  
301 leaf beetle on the trichome dimorphism of *A. halleri*. This study consists of three  
302 specific approaches (Fig. 2). The first stage is to identify candidate mechanisms of  
303 frequency-dependent damage between hairy and glabrous plants. In the second stage,  
304 manipulative experiments are conducted to test fitness consequence and to address  
305 whether AE against the leaf beetle results in negative frequency-dependent selection  
306 between the two morphs. The final step is to link the experimental findings with field  
307 observations by modeling mechanisms that govern plant population dynamics. These  
308 three steps will provide comprehensive evidence that AE play a stabilizing role in plant  
309 defense polymorphism.

310

### 311 2-1. Candidate mechanisms of AE

312 Behavioral response of herbivores to multiple resources can be a mechanism of AE (e.g.,

313 Bergvall *et al.* 2006; Verschut *et al.* 2016; Hahn & Orrock 2016). Choice assays are  
314 often conducted to test the candidate mechanism of AE (Tahvanainen & Root 1972;  
315 White & Whitham 2000; Rand 2003; Hahn & Orrock 2016). Several studies  
316 documented that the feeding preferences of herbivores depend on the presence or  
317 relative frequencies of multiple resource types (Chandra & Williams 1983; Cottam  
318 1985; Behmer *et al.* 2001; Bergvall & Leimar 2005; Janz *et al.* 2005), but evidence  
319 regarding such a frequency-dependent herbivory on natural variation in a plant defense  
320 trait is limited.

321           My collaborators and I have conducted a four-way choice experiment  
322 manipulating frequencies of hairy and glabrous leaf discs (Fig. 2b: Sato *et al.* 2014).  
323 Adults of the brassica leaf beetle *P. brassicae* avoided hairy leaves when hairy leaves  
324 were rare, whereas the adults did not show a feeding preference when hairy leaves  
325 became abundant (Sato *et al.* 2014). The adult preference for glabrous leaves was also  
326 observed in a binary choice experiment using a *gll* mutant of the model plant species  
327 *Arabidopsis thaliana* (Sato 2016). This result from a single-gene mutant provides strong  
328 proof for the resistance function of the *GLI* gene against *P. brassicae*. Furthermore,  
329 when adult beetles were starved or preconditioned with glabrous diets, they still  
330 preferred glabrous plants (Sato & Kudoh 2016b). Contrarily, when adults were  
331 preconditioned with hairy diets, this treatment weakened their feeding preference for  
332 glabrous diets (Sato & Kudoh 2016b). The circumstantial evidence suggests that  
333 sequential experience may trigger a non-random response of adult beetles to hairy and  
334 glabrous diets. While adult beetles fed on both hairy and glabrous leaves at similar  
335 levels under no-choice conditions, larvae fed on hairy diets grew slower than those on  
336 glabrous diets. This indicates that hairy diets are suboptimal for *P. brassicae*.

337           The foraging behaviors of *P. brassicae* were then modeled to reveal the  
338 mechanism underlying the pattern of leaf damage to hairy and glabrous plants. Given  
339 that *P. brassicae* is flightless and incapable of selecting plants among patches, the  
340 optimal diet choice (Charnov 1976) is assumed to be a mechanism by which rare  
341 suboptimal diets (i.e., hairy leaves) are less consumed under the small enclosed  
342 condition. My collaborators and I modified the original model of optimal diet choice to  
343 consider imperfect host recognition by *P. brassicae*, and then fitted the model to the  
344 laboratory experiment data of leaf damage under the contrasting frequency of hairy and  
345 glabrous plants (Sato *et al.* 2017). As a result of the parameter estimation, it was  
346 estimated that decreased energy intake and increased handling time for the leaf beetles  
347 accounted for the frequency-dependent damage on hairy plants (Fig. 2c). Hairy plants  
348 were less damaged when glabrous plants became abundant, indicating associational  
349 resistance for hairy plants. Contrarily, damages to glabrous plants weakly depended on  
350 the frequency of two plant morphs. These findings indicate that the optimal diet choice  
351 by *P. brassicae* can be a determinant of the pattern of AE between hairy and glabrous  
352 plants.

353

#### 354 *2-2. Manipulative experiment showing a rarity advantage in plant defense and fitness*

355 Even if herbivores feed on hairy and glabrous plants in a frequency-dependent manner,  
356 it is still unknown whether this candidate mechanism leads to frequency-dependent  
357 selection on the trichome dimorphism. Specifically with regard to the brassica leaf  
358 beetle *P. brassicae*, it seems unlikely that herbivore preference alone generates negative  
359 frequency-dependent selection, as they did not avoid rare glabrous diets (Sato *et al.*  
360 2014: Fig. 3a). However, game theoretical models suggest that, if plants have defense

361 traits at the cost of their growth or fecundity, the benefit from associational effects  
362 allows defended and undefended plants to coexist (Augner *et al.* 1991). This hypothesis  
363 was tested using a laboratory and semi-field experiment manipulating the morph  
364 frequency and presence/absence of *P. brassicae* (Fig. 2b; Sato & Kudoh 2016b; Sato &  
365 Kudoh 2017b).

366 Figure 3 shows a schematic explanation of a mechanism by which conspecific  
367 AE could be a mechanism for the maintenance of trichome dimorphism. Consistent with  
368 the feeding preference assay (Sato *et al.* 2014), hairy plants were less damaged than  
369 glabrous plants only when the hairy ones were rare (Fig. 3a: Sato & Kudoh 2016b; Sato  
370 & Kudoh 2017b). This explains the rarity advantage for hairy plants in defense and  
371 fitness (Fig. 3a, c). At the end of the experiments, both the hairy and glabrous plants had  
372 a rarity advantage in fitness in the presence of beetles (Fig. 3c: Sato & Kudoh 2016b;  
373 Sato & Kudoh 2017b), while in the absence of herbivory the glabrous plants had a  
374 higher growth and fecundity (Fig. 3b: Sato & Kudoh 2016b; Sato & Kudoh 2017b).  
375 Because the benefit of associational effects to hairy plants no longer exists under the  
376 hairy-abundant condition (Fig. 3a), the cost of defense can be detected when hairy  
377 plants become abundant (Fig. 3c). Of note, these fitness consequences were consistently  
378 observed for both sexual and asexual reproduction, namely the number of flowers and  
379 clones, of *A. halleri* (Sato & Kudoh 2017b). These experimental findings indicate that,  
380 together with the defense-growth tradeoff (Fig. 3c), conspecific AE against the leaf  
381 beetle (Fig. 3a) lead to negative frequency-dependent selection between hairy and  
382 glabrous plants (Fig. 3b) (Sato & Kudoh 2017b).

383

384 *2-3. Field patterns of herbivory and polymorphism dynamics*

385 The set of experiments illustrated conspecific AE that can maintain the trichome  
386 dimorphism of *A. halleri*, but it is still unknown whether these experimental findings  
387 represent AE in the wild. Next, field observations on plant damage and demography  
388 were conducted in a natural population in which the brassica leaf beetle *P. brassicae*  
389 was a major herbivorous insect on *A. halleri* (Fig. 2b). Because *P. brassicae* is flightless  
390 and the *A. halleri* population has a fragmented structure, AE is assumed to occur on a  
391 small spatial scale. Thus, my collaborators and I tracked the aggregated structure of the  
392 plants (referred to hereafter as “patch”) for four years in the field.

393 Hairy plants received less damage as the frequency of glabrous plants increased  
394 in a series of plant patches (Sato *et al.* 2014; Sato & Kudoh 2017b). In contrast, leaf  
395 damage on glabrous plants showed no significant and consistent relationship with the  
396 frequency of glabrous plants in a patch (Sato *et al.* 2014; Sato & Kudoh 2017b). This  
397 pattern of leaf damage was consistent with the results of a feeding assay. It was also  
398 noteworthy that the relative fitness advantage of hairy plants as shown by flower  
399 production in the presence of beetles increased with increasing frequencies of glabrous  
400 plants (Sato & Kudoh 2017b). This pattern in the flower production agrees with the  
401 manipulative experiment detecting negative frequency-dependent selection only in the  
402 presence of *P. brassicae* (Fig. 3c). These findings support the relevance of  
403 frequency-dependent herbivory and selection in the natural population.

404 A multi-year field survey also found that rarer morphs increased in frequency  
405 in a series of plant patches over time (Sato & Kudoh 2017b). Statistical modeling was  
406 used to reveal the mechanisms underlying the temporal dynamics of morph frequency in  
407 the field (Sato *et al.* 2017). Based on the aforementioned theory of optimal diet choice,  
408 my collaborators and I have modelled the effects of local-scale herbivory and spatial

409 structure on plant demography and then fitted the model to the observed changes in the  
410 number of hairy and glabrous plants over four years. Based on a model selection  
411 procedure, it was revealed that optimal diet choice by *P. brassicae* contributes to the  
412 polymorphism dynamics observed in the field (Sato *et al.* 2017). The estimated  
413 magnitude of interpatch dispersal suggested that long-distance seed dispersal was rare  
414 and clonal reproduction largely contributed to the plant population growth. According to  
415 the estimated parameters of antiherbivore defense, rarer morphs had higher fitness and  
416 increased in frequency over time when the herbivore diet choice and cost of plant  
417 defense were both involved (Fig. 2c). This joint approach using the optimality modeling  
418 and field data strongly supports the hypothesis that AE against the leaf beetle promotes  
419 the maintenance of the trichome dimorphism via negative frequency-dependent  
420 selection.

421           In contrast to the constant density in the manipulative experiment (Fig. 3), not  
422 only frequency but also the number of plants varies among field patches. However, the  
423 effects of plant density on damage were much less significant than that of frequency,  
424 and its magnitude and direction were inconsistent among the study years (Sato *et al.*  
425 2014; Sato & Kudoh 2017b). The resource concentration or delusion of *A. halleri* for *P.*  
426 *brassicae* was tested in more detail using the field data on the leaf damage and number  
427 of *A. halleri* plants (Sato *et al.* 2017). If these two effects of plant density occur, there is  
428 a non-linear relationship between the total amount of herbivory load and the total  
429 number of plants within patches (Hambäck *et al.* 2014). In *A. halleri*, however, the total  
430 amount of leaf damage increased linearly with the total number of plants in a patch  
431 (Sato *et al.* 2017), presumably because the plant density in the field was too low to  
432 reach a peak of resource loss by herbivory. Thus, at least for the temporal scales

433 observed, the field survey could not detect a significant role of plant density in AE  
434 against the leaf beetles.

435 Overall, the multiple lines of evidence have shown that conspecific AE  
436 mediated by *P. brassicae* lead to negative frequency-dependent selection and thereby  
437 maintain the trichome dimorphism in a natural *A. halleri* population. If hairy plants are  
438 rare, they can escape from leaf beetles and increase in frequency. On the other hand, if  
439 glabrous plants become rare, they outcompete hairy plants without any costs of  
440 trichomes. Consequently, this negative feedback loop prevents rare morphs from going  
441 extinct (Fig. 4). To date, negative frequency-dependent selection has been reported for  
442 plant species interacting with pollinators (Gigord *et al.* 2001) and pathogens  
443 (Antonovics & Ellstrand 1984; Brunet & Munht 2000), but it has not yet been  
444 demonstrated in plant defense against herbivores. The case study on *A. halleri*-*P.*  
445 *brassicae* interaction provides the first example to illustrate that conspecific AE drive  
446 negative frequency-dependent selection on an antiherbivore defense trait.

447

### 448 **3. Perspective**

449 Genetic polymorphisms are commonly observed in plant defense traits (Hughes 1991;  
450 Elle *et al.* 1999; Wise *et al.* 2009; Bernhardsson *et al.* 2013) and not limited to  
451 *Arabidopsis* trichomes. For example, the genetic basis of cyanogenesis in white clover  
452 *Trifolium repens* (Fabaceae) is well studied (Hughes 1991). Theory related to  
453 herbivore-mediated frequency-dependent selection was applied to geographical  
454 variation in a cyanogenesis polymorphism (Till-Bottraud & Gouyon 1992), but the  
455 existence of frequency-dependent selection has not been tested experimentally.  
456 Dimorphism of the glandular (sticky) and non-glandular (velvety) *Datura wrightii*

457 (Solanaceae) is inherited in the Mendelian fashion (van Dam *et al.* 1999). This chemical  
458 defense utilizing glandular exudates is costly for plant fitness (Elle *et al.* 1999), but it  
459 remains unknown why these sticky and velvety plants can coexist (Elle & Hare 2000).  
460 The genetic basis of glucosinolates, a major secondary metabolite of Brassicales, has  
461 been intensively studied. Genetic polymorphisms are observed in physiological  
462 pathways in glucosinolate biosynthesis and result in diverse profiles among natural  
463 *Arabidopsis* accessions (Chan *et al.* 2010). Conspecific AE have not yet been examined  
464 in these defense traits but could provide insights into mechanisms for the maintenance  
465 of antiherbivore defense polymorphism within a plant population.

466         We should note here that not only AE but also other factors may shape genetic  
467 variation in antiherbivore defenses. It has been hypothesized that genetic variation may  
468 be maintained by spatiotemporal fluctuation in selection pressure and stochastic gene  
469 flow among populations (e.g., Levene 1953; Turelli *et al.* 2001; but see Spichtig &  
470 Kawecki 2004). The present paper has focused on local-scale AE so far, but to what  
471 spatial and temporal scales can AE contribute to the maintenance of polymorphism in  
472 plant defense? In the final chapter, I discuss a spatial and temporal perspective of  
473 conspecific AE and the maintenance of plant defense polymorphism. Concerning the  
474 spatial scale, several researchers discussed interspecific AE at a landscape scale  
475 (reviewed by Barbosa *et al.* 2009). The geographic mosaic of coevolution (Thompson  
476 2005) proposes a landscape perspective of plant–insect interactions, and this spatial  
477 point of view is applied to geographic variation in plant defense (e.g., Berenbaum &  
478 Zangerl 1998; Zangerl & Berenbaum 2003; Toju *et al.* 2011; Bernhardsson *et al.* 2013).  
479 In particular to temporal dynamics, the concept of eco-evolutionary dynamics has  
480 recently suggested interactions between plant genetic variation and herbivore abundance

481 (Turcotte 2011; Turcotte *et al.* 2013; Agrawal *et al.* 2013). Because not only frequency-  
482 but also density-dependent selection is a key aspect of AE (Table 1), conspecific AE  
483 may provide insights into how rapid evolution in plant defenses affects the population  
484 dynamics of plants and herbivores.

485

486 *3-1. Can AE create a source population for geographical variation in plant defense?*

487 Existing theory suggests that negative frequency-dependent selection contributes to  
488 geographic cline in a polymorphism, as it makes morph frequency intermediate rather  
489 than expected by the results of disruptive selection alone (Clarke 1966; Endler 1977;  
490 Till-Bottraud & Gouyon 1992). Till-Bottraud & Gouyon (1992) applied this prediction  
491 to explain a cyanogenesis polymorphism along an altitudinal cline in white clover.  
492 However, herbivory regimes usually have more complex spatial patterns than a cline,  
493 involving differences in herbivore species (Hare & Elle 2002) and the presence of other  
494 host plants (Zangerl & Berenbaum 2003). Thus, plant–herbivore interactions are  
495 expected to result in complex geographic variation rather than a simple cline in a plant  
496 defense polymorphism.

497         The geographic mosaic of coevolution proposes a conceptual framework for  
498 investigating the complex metapopulation structure of local adaptation/maladaptation  
499 (Thompson 2005). Specifically, the theory of geographic mosaic of coevolving  
500 polymorphism hypothesizes that disruptive and balancing selection jointly shape  
501 complex patterns of host defense polymorphism (Thompson 2005). In plant–herbivore  
502 interactions, Berenbaum and Zangerl (1998) presumed that intense attacks by parsnip  
503 webworm *Depressaria pastinacella* on abundant chemotypes of a wild parsnip  
504 *Pastinaca sativa* (Apiaceae) led to negative frequency-dependent selection and thus

505 favored the intermediate chemotype frequency. Given that AE should occur among  
506 individual plants in close vicinity, this may alter spatial patterns of plant defense only at  
507 a local scale. More generally, the metapopulation framework assumes the existence of  
508 source and sink populations of local adaptation (Thompson 2005). When AE favors  
509 polymorphism within a local population, this polymorphic population could be a source  
510 of polymorphism on a metapopulation scale via gene flow (Fig. 5).

511         Indeed, herbivore regimes and frequencies of hairy and glabrous plants vary  
512 among natural populations of *Arabidopsis halleri* subsp. *gemmaifera*, where AE do not  
513 occur against all herbivorous insects on *A. halleri*. Sato and Kudoh (2015) found that  
514 AE were detectable when *A. halleri* interacted with slow-moving herbivores such as *P.*  
515 *brassicae*. AE tend to be obscured when plants are attacked by flying herbivores such as  
516 the green-veined white butterfly *Pieris napi* and cabbage sawfly *Athalia infumata*,  
517 although trichomes themselves can prevent herbivory by these herbivores (Sato &  
518 Kudoh 2015). Field surveys revealed that frequencies of glabrous plants declined as  
519 herbivory pressure became intense (Sato & Kudoh 2017a). Hairy-monomorphic  
520 populations were discovered where white butterflies were frequently observed. Despite  
521 experiencing the severest herbivory pressure, however, a population where *P. brassicae*  
522 was predominant still had an intermediate morph frequency (Sato & Kudoh 2017a).  
523 Furthermore, a proximate population was also polymorphic despite the prevalence of  
524 white butterflies (Sato & Kudoh 2017a). These observations support the hypothesis that  
525 herbivore-mediated frequency-dependent selection sustains polymorphism, which can  
526 be a source of genetic variation in proximate populations (Fig. 5b). Further questions  
527 remain about how conspecific AE contributes to the maintenance of polymorphism at  
528 the metapopulation level.

529

530 3-2. Do AE affect population dynamics via the maintenance of defense polymorphism?

531 A growing number of studies have shown that herbivores drive the evolution of plant  
532 defense on ecological time scales (Zangerl & Berenbaum 2008; Agrawal *et al.* 2012;  
533 Fukano & Yahara 2012; Sakata *et al.* 2014). Recent studies have also shown that such  
534 evolutionary changes in a host plant species alter the population dynamics and  
535 community structures of herbivores (Crutsinger *et al.* 2006; Johnson *et al.* 2006;  
536 Turcotte *et al.* 2011; Agrawal *et al.* 2013), which may in turn affect plant density. Now  
537 that conspecific AE is known to maintain polymorphism in plant defense, it is possible  
538 that such a sustained polymorphism affects eco-evolutionary dynamics in  
539 plant–herbivore interactions. In the context of AE, Underwood *et al.* (2014) advocate  
540 that manipulating both the density and frequency of multiple plant species is important  
541 for complete understanding of the long-term coexistence of multiple plant species under  
542 AE (as introduced in Section 1-2.). When this notion is applied to polymorphisms  
543 within a plant species, AE may play an important role not only through frequency- but  
544 also through density-dependent selection in plant defense.

545         Once the mechanisms of frequency- and/or density-dependent selection are  
546 experimentally shown, it is possible to estimate the probability of coexistence from the  
547 experimental data (e.g., Damgaard 1998; Inouye 2001; Miller & Rudgers 2014) or to  
548 predict temporal dynamics in the field (Kim *et al.* 2013; Le Rouzic *et al.* 2015) based on  
549 a given theoretical model. This modeling approach, combined with empirical data, will  
550 be a powerful tool to reveal how AE contribute to eco-evolutionary dynamics in  
551 plant–herbivore interactions. For example, Sato *et al.* (2017) took such a modeling  
552 approach to *A. halleri*–*P. brassicae* interactions although the modeling was limited in

553 predicting long-term interactions between the evolution of plant defenses and herbivore  
554 population dynamics. Specifically, the magnitude of herbivory in the current year was  
555 not clearly correlated with that in the previous year or with the frequency of hairy plants  
556 (Sato *et al.* 2017). If long-term data on herbivory and population dynamics are obtained,  
557 the modeling approach might enable us to predict how the existence of polymorphism  
558 affects herbivory pressure and in turn alters the morph frequency within a plant species.

559

### 560 *3-3. Concluding remarks*

561 Associational effects, or neighbor effects, are remarkable phenomena in plant–herbivore  
562 interaction, but evidence for conspecific AE is still limited. By focusing on polymorphic  
563 defense traits within a plant species, the present review highlights the potential  
564 importance of conspecific AE in maintaining polymorphism in an antiherbivore defense  
565 trait. The multiple lines of evidence from *P. brassicae*–*A. halleri* interaction show that  
566 conspecific AE maintains antiherbivore defense polymorphism via negative  
567 frequency-dependent selection. Future studies should focus on the stabilizing role of AE  
568 in genetic variation in plant defense, as diverse plant species possess considerable  
569 variation in terms of antiherbivore defense traits.

570

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580

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912 **Table 1.** Examples of associational effects that cause frequency- and/or density-dependent herbivory between plant species/genotypes.

Factor	Article	Study system		Neighborhood composition
		Plant (or Resource)	Herbivore (or Consumer)	
Density	Rand (2003)	<i>Atriplex patula</i> and <i>Salicornia europaea</i>	Polyphagous beetle <i>Erynephala maritima</i>	Presence/absence of the other species with low/high density
	Russell & Louda (2005)	<i>Cirsium canescens</i> and <i>C. undulatum</i>	Florivorous weevil <i>Rhinocyllus conicus</i>	Density of the other species in natural populations
Frequency	Bergvall <i>et al.</i> (2006)	Low- and high-tannin food	Fallow deer <i>Dama dama</i>	Rare or abundant type A against B (= 2 frequency conditions)
	Wise <i>et al.</i> (2009)	Candy-cane and erect-stem genotype of <i>Solidago altissima</i>	Oligophagous gall-fly <i>Eurosta solidaginis</i>	Rare or abundant type A against B (= 2 frequency conditions)
	Sato & Kudoh (2016b)	Hairy and glabrous genotype of <i>Arabidopsis halleri</i> subsp. <i>gemmaifera</i>	Oligophagous beetle <i>Phaedon brassicae</i>	Rare or abundant type A against B (= 2 frequency conditions)
	Garrido <i>et al.</i> (2016)	Resistant and tolerant genotype of <i>Datura stramonium</i>	Oligophagous beetle <i>Lema daturaphila</i>	Rare, even, and abundant type A against type B (= 3 frequency conditions)
Both	Kim & Underwood (2015)	<i>Solidago altissima</i> and <i>Solanum carolinense</i>	(incl. multiple species)	Response surface design of 4 frequency and 4 density conditions
	Hahn & Orrock (2016)	<i>Solidago nemoralis</i> and <i>S. odora</i>	Grasshoppers	Rare, even, and abundant type A against type B (= 3 frequency conditions) at high/low densities.
	Verschut <i>et al.</i> (2016)	Balsamic vinegar solutions	<i>Drosophila melanogaster</i>	Different resource concentration (2 levels) with 3 or 5 frequency conditions

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914 **Figure legends**

915 **Figure 1.** Stepwise approach to reveal causes and consequences of associational effects  
916 (AE) on plant coexistence. The reference indicates studies comprising a focal approach.  
917 Details of the selected references are shown in Appendix.

918

919 **Figure 2.** Comprehensive test for the maintenance of the trichome dimorphism of  
920 *Arabidopsis halleri* subsp. *gemmifera* mediated by associational effects against a leaf  
921 beetle *Phaedon brassicae*. **(a)** Photographs of plants and insects; **(b)** Empirical  
922 approaches from laboratory experiments to field observation; **(c)** Modeling herbivory,  
923 plant fitness, and polymorphism dynamics. Insets in the panel **(c)** are modified from  
924 Sato *et al.* (2017).

925

926 **Figure 3.** Mechanism through which associational effects and a cost of defense jointly  
927 cause negative frequency-dependent selection between hairy and glabrous plants. Panels  
928 **(a)**, **(b)** and **(c)** represent differences in herbivory, intrinsic fitness, and realized fitness  
929 between hairy and glabrous plants, respectively.  $H > G$  and  $H < G$  indicates  
930 hairy-abundant and glabrous-abundant condition, respectively.

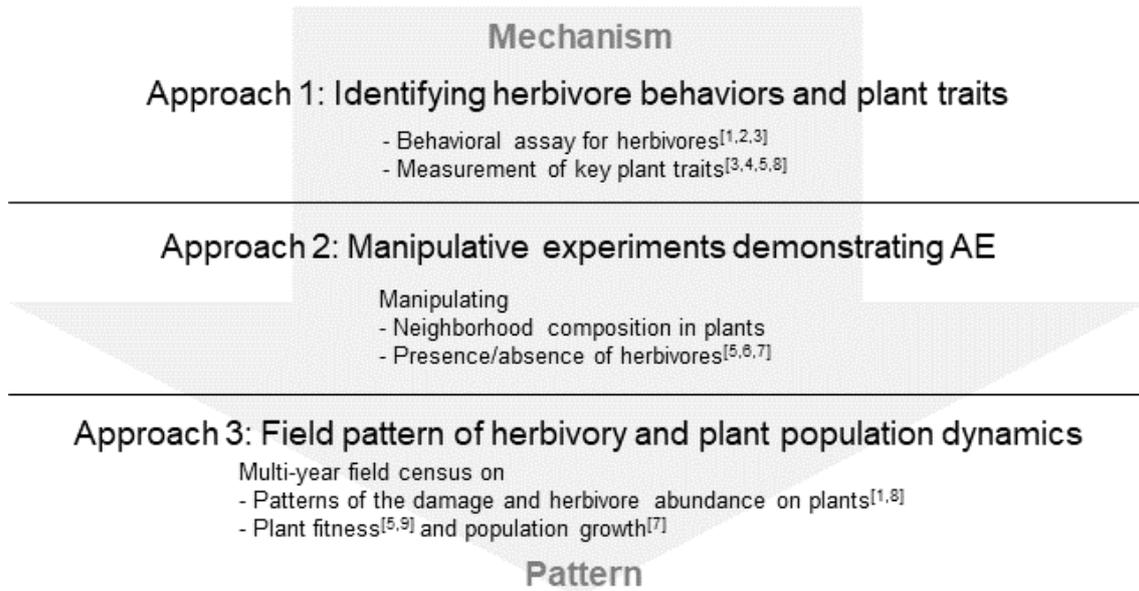
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932 **Figure 4.** Evolutionary dynamics wherein the trichome dimorphism of *Arabidopsis*  
933 *halleri* subsp. *gemmifera* can be maintained by the herbivore-mediated  
934 frequency-dependent selection. Frequency-dependent feeding preference by herbivores  
935 and plant defense-growth tradeoff results in a negative feedback loop that allows rarer  
936 plant morphs to invade into a population.

937

938 **Figure 5.** Specific hypothesis for how local-scale associational effects (AE) contribute  
939 to geographic variation in plant defense via the maintenance of source population in a  
940 polymorphism. **(a)** Relationship between the herbivory pressure and morph-frequency  
941 in the presence (solid line) and absence (dashed line) of AE. **(b)** Contribution of a  
942 source polymorphic population created by AE to the maintenance of polymorphism at a  
943 metapopulation scale. Grey and white square represents the region of intense and  
944 moderate herbivory, respectively. White arrows represent gene flow from a source to  
945 nearby populations.

946 **Figure 1**



[1] Tahvanainen & Root (1972); [2] White & Whitham (2000); [3] Bergvall *et al.* (2006); [4] Barton & Browers (2006); [5] Agrawal (2004); [6] Rand (2003); [7] Callaway *et al.* (2005); [8] Russell & Louda (2005); [9] Karban & Maron (2002)

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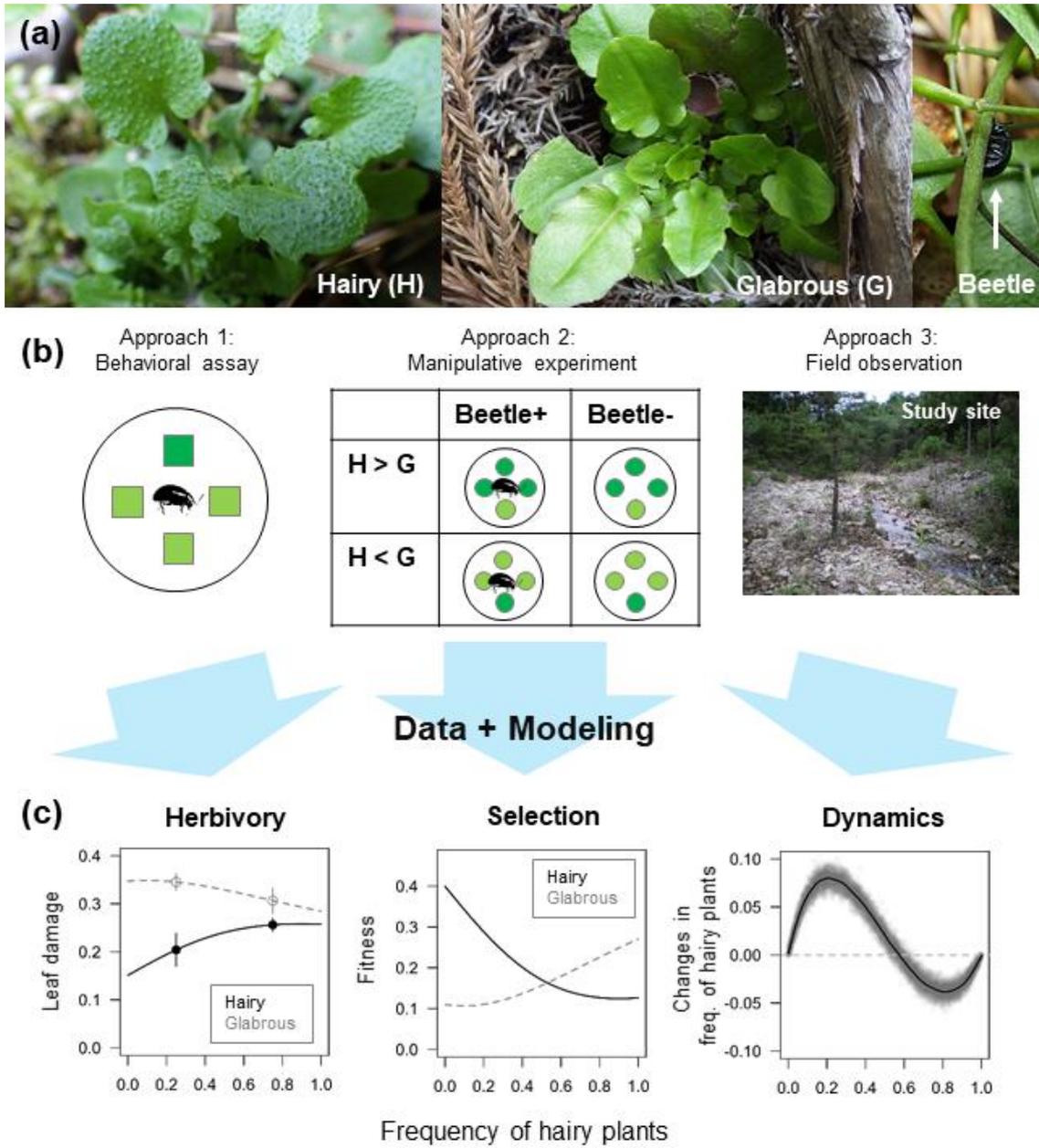
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961 **Figure 2**



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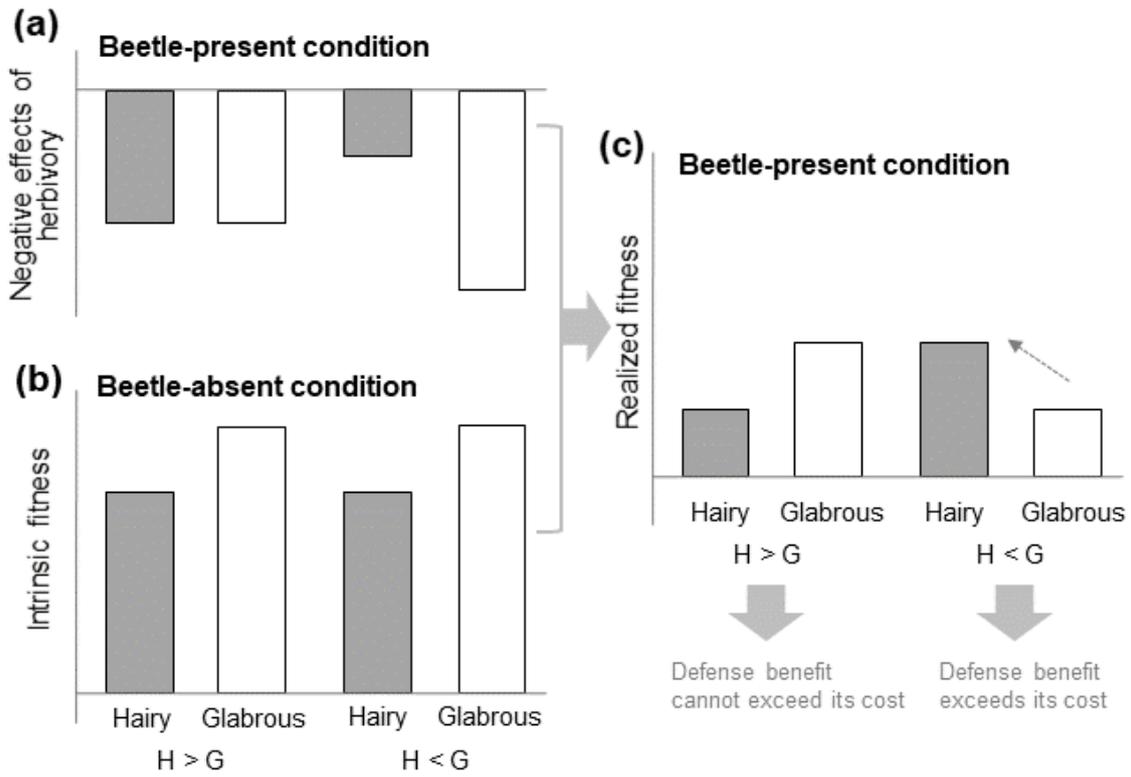
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968 **Figure 3**



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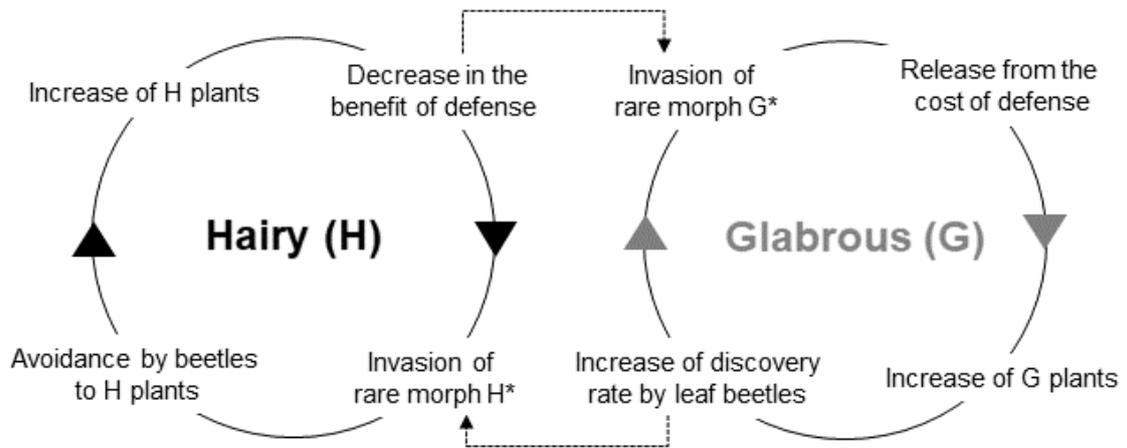
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982 **Figure 4**



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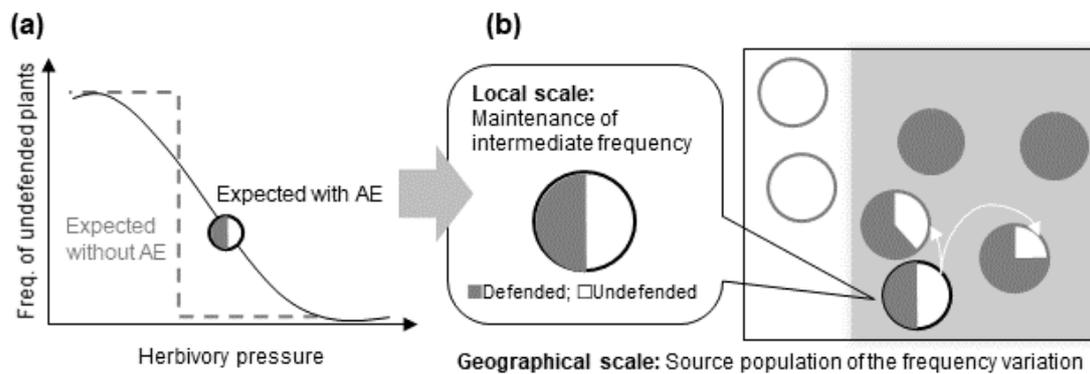
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1000 **Figure 5**



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**Appendix.** Selected examples of associational effects in plant-herbivore interaction. Study system (plants and herbivores), design (experimental design and spatial scales, reciprocal data or not), and results (measurement, outcome, and putative mechanism) are summarized.

Reference	Study system			Study design			Results		
	Herbivore	Plant species	Plant traits	Experimental design	Scale	Reciprocal or not	Measurement	Outcome	Mechanism
Tahvanainen & Root (1972)	A specialist flea beetle ( <i>Phyllotreta cruciferae</i> )	<i>Brassica oleracea</i> and tomato or tobacco	Odor (shown by a choice assay)	Monoculture of <i>B. oleracea</i> and polyculture of three species. The tomato and tobacco was added to the equal density of <i>B. oleracea</i> in the polyculture.	Within-patch	NA for tomato and tobacco	The number of beetles per plant and plant biomass for <i>B. oleracea</i>	AR: The number of beetles per plant was higher, and plant biomass decreased under the monoculture.	Odor cues for herbivores
Rausher (1981)	A specialist butterfly ( <i>Battus philenor</i> )	<i>Aristolochia reticulata</i> and others species in near vegetation	Unidentified	0.5 m-radius vegetation surrounding an <i>A. reticulata</i> individual was removed. Non-removed patches were assigned to the control.	Within-patch	NA for counterparts	Oviposition per <i>A. reticulata</i> plant	AR: Isolated plants suffer more oviposition, butterflies spend more time in the isolated patch.	Searching efficiency of herbivores
Risch (1981)	Mono- Oligo-, Polyphagous beetles (6 species)	Corn ( <i>Zea mays</i> ), beans ( <i>Phaseolus vulgaris</i> ), and squash ( <i>Cucurbita maxima</i> )	Unidentified	Intercropping one crops into another; monoculture, diculture triculture for 3 species (= 7 conditions)	Within-patch	Reciprocal	No of beetles per plant	AR: The beetle abundance reduced under polycultic conditions except for a squash-bean diculture	Resource concentration (plant density)
Hay (1986)	Generalist fishes	Multiple species of Marine algae	Unidentified	Solitary or paired culture in a closed cage	Within-patch	Reciprocal	Within-patch: predation risk by fishes	AR for palatable algae to generalist fishes	Physical attachments to unpalatable algae
White & Whitham (2000)	Fall cankerworm ( <i>Alsophila pometaria</i> ).	Cotton ( <i>Populus angustifolia</i> × <i>P. fremontii</i> ), and box elder ( <i>Acer negundo</i> )	Unknown, (but a choice test showed that the cankerworm prefer to the box elder).	Transplanting juvenile cottons to three conditions: Nearby box elder, Nearby matured cotton, Stand alone.	Within-patch	NA for the box elder	Larvae density per shoot of the juvenile cotton	AS: Cotton suffered from the higher density nearby a heterospecific counterpart than nearby conspecifics or stand alone.	Herbivore dispersal from palatable to unpalatable plants
Karban & Maron (2002)	Generalist grasshoppers (incl. multiple species)	Sagebrush ( <i>Artemisia tridentata</i> ) and tobacco ( <i>Nicotiana attenuata</i> )	Volatile chemical	Tobacco was transferred near damaged or intact sagebrush.	Within-patch	NA for Sagebrush	The number of flowers and capsules per tobacco	AR: tobacco produced more flowers and capsules nearby damaged sagebrush.	Volatile communication between plants
Hambäck <i>et al.</i> (2000)	A specialist leaf beetle ( <i>Galerucella californiensis</i> )	An aromatic shrub ( <i>Myrica gale</i> ) and a perennial herb ( <i>Lythrum salicaria</i> )	Unidentified	Stand-alone or within- <i>M. gale</i> patch condition for <i>L. salicaria</i>	Within-patch	NA for <i>M. gale</i>	Oviposition, leaf damage, seed production per individual <i>L. salicaria</i> .	AR: <i>L. salicaria</i> incurred less oviposition and damage, and produce more seeds nearby <i>M. gale</i>	Odor or visual cypsis. Predator abundance did not differ between the two conditions.
Rand (2003)	A generalist leaf beetle ( <i>Erynephalam aritima</i> )	Two forbs, <i>Atriplex patula</i> var. <i>hastata</i> and <i>Salicorniae uropaea</i>	Unidentified (but feeding preference was shown by a choice test).	The presence/absence of herbivores for 3 conditions: <i>Atriplex</i> alone with low density, <i>Atriplex</i> alone with high density, <i>Atriplex</i> with <i>Salicornia</i>	Within-patch	NA for <i>Salicornia</i>	Leaf damage, the number of larvae, and survival (%) of plants.	AS: <i>Atriplex</i> suffered more leaf damage and less survival nearby <i>Salicornia</i>	Diet-mixing by generalist herbivore
Agrawal (2004)	A specialist beetle ( <i>Tetraopes tetraophthalmus</i> )	a milkweed ( <i>Asclepias syriaca</i> ) and a grass ( <i>Liriomyza asclepiadis</i> )	Trichome, Latex, Nitrogen contents	Grasses or beetles were added to a potted milkweed: 4 treatments of Control, Grass+, Beetle+, and Grass&Beetle+. Grass alone, Grass&Milk&Beetle, Grass&Milk was also set.	Within-patch	Grass biomass data: Grass alone = Grass&Milk&Beetle > Grass&Milk	Milkweed biomass, fruit production, fruit mass; and grass biomass	AS: milkweed biomass and fecundity was Control ≈ Grass+ > Beetle+ > Grass&Beetle+.	Dispersal from primary host plants to secondary ones.

Callaway <i>et al.</i> (2005)	Mammals	Four unpalatable herbs ( <i>Cirsium</i> , <i>Veratrum</i> , <i>Luzula</i> , <i>Alchemilla</i> ) and two palatable herbs ( <i>Anthoxanthum</i> , <i>Phleum</i> )	Spines (for <i>Cirsium</i> ) or toxin (for <i>Veratrum</i> )	Four focal species × within/outside grazing fence × exclusion of <i>Cirsium</i> or <i>Veratrum</i> = 24 treatments	Within-patch	NA for <i>Cirsium</i> and <i>Veratrum</i>	Annual growth rates of four plant species.	AR: increased growth rate of two palatable species, no increase of growth rate of unpalatable two species nearby <i>Cirsium</i> and <i>Veratrum</i>	avoidance by grazers to plant spines and toxins.
Russell & Louda (2005)	Florivorous weevil, <i>Rhinocyllus conicus</i>	Two congeneric natives, <i>Cirsium canescens</i> and <i>C. undulatum</i>	Plant phenology	> 10-yrs field census in 13 sites where the two plant species co-occur	Within-site	NA for <i>C. canescens</i>	Proportion of weevils and their eggs on <i>C. undulatum</i> .	AR for <i>C. undulatum</i> : The proportion of weevils and eggs was negatively correlated with the number of <i>C. canescens</i> .	Phenological synchronicity of flowering between the two species.
Bergvall <i>et al.</i> (2006)	Fallow deer ( <i>Dama dama</i> )	Unknown. Synthetic food containing different tannin levels	Chemical (Tannin)	Bad-patch and good-patch contains 7:1 and 1:7 of high:low tannin buckets, respectively. Single- and group-foraging of deers were also tested.	Within and between patch	Reciprocal	Log-consumption per bucket (g).	AS for low-tannin buckets: More consumption on low-tannin buckets in the bad-patch than those in the good-patch. AR for high-tannin buckets: Less consumption on high-tannin buckets in the good-patch than those in the bad-patch. AS for <i>P. lanceolata</i> ?: <i>P. lanceolata</i> surrounded by heterospecific plants produced less defensive chemicals compared to those surrounded by conspecifics.	within- and among-patch foraging behavior of mammalian herbivores.
Barton & Bowers (2006)	None (only defense phenotypes were evaluated)	Interspecific: two congeners, <i>Plantago lanceolata</i> and <i>P. major</i>	Chemical (Iridoid glucoside)	2 species × (conspecific, heterospecific, no neighbor) × 2 harvesting time points = 12 conditions	Within-patch	Reciprocal	Within-patch: % Dry Weight of Iridoid Glycosides		Plant trait change via direct competition between plants.
Le Guigo <i>et al.</i> (2012)	A specialist aphid ( <i>Brevicoryne brassicae</i> ) and generalist aphid ( <i>Myzus persicae</i> )	Three <i>Brassica</i> species and tomato ( <i>Solanum lycopersicum</i> )	Chemical (glucosinolate?)	A <i>Brassica oleracea</i> individual was surrounded by 6 conspecific or heterospecific individuals (= 7 treatments in total).	Within-patch	Reciprocal	The number of aphids on a focal <i>B. oleracea</i> , compared between conspecific and interspecific conditions	AS when a herbivore is specialist; AR when a herbivore is generalist.	Chemical attraction or repulsion
Castagneyrol <i>et al.</i> (2013)	Leaf-chewers (beetles, caterpillars, and grasshoppers) or Leaf-miners (incl. 5 species)	<i>Betula pendula</i> , <i>Quercus robur</i> , <i>Quercus pyrenaica</i> , <i>Quercus ilex</i> , <i>Pinus pinaster</i>	Plant height, and leaf area, N and P contents, leaf life span	31 possible mono- and poly-culture combinations of 1~5 plant species	Within/Between patch	Relative values of two species (for plant height data)	Leaf area loss (by chewers) and leaf-miner abundance at plot and individual scale.	AR against leaf-miners: no AR against leaf-chewers	Variation in leaf traits and apparency (plant height)
Kim & Underwood (2015)	3 species of specialists (for <i>S. carolinense</i> ), and 3 species of generalist herbivores	<i>Solanum carolinense</i> , <i>Solidago altissima</i>	Unidentified	Response surface design (1, 6, 12, 18 total plant number with 4 frequencies of <i>S. altissima</i> )	Within-patch	Reciprocal (shown in the supplement)	Leaf damage (%) of individual plants	AS for <i>S. carolinense</i> and damage was positively density-dependent.	Unknown (but those depending on the plant density and frequency)

Abbreviation: AR; Associational resistance, AS; Associational susceptibility, NA; No information available