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## **Optimal foraging by herbivores maintains polymorphism in defence in a natural plant population**

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## SUMMARY

1. Many species of plants and animals exhibit polymorphism for defensive traits. Adaptive foraging by natural enemies has long been hypothesized to maintain such polymorphism, but this has not been clearly demonstrated in a natural prey or host population.
2. The purpose of this study was to address whether the brassica leaf beetle *Phaedon brassicae* promotes the maintenance of defence polymorphism in the trichome-producing (hairy) and trichomeless (glabrous) morphs of *Arabidopsis halleri* subsp. *gemmifera*. Here, we modelled foraging behaviours of herbivores and demography of the host plant. Then, we estimated the model parameters based on the likelihood of observed data from a natural *A. halleri* population.
3. The patterns of leaf damage to hairy and glabrous plants were well explained when we presumed the optimal diet choice by *P. brassicae*. The observed dynamics in the plant number and morph frequency were well supported by the model with the estimated parameter values. Our numerical analysis showed that the optimal diet choice by *P. brassicae* caused a negative frequency-dependent selection on trichome dimorphism. The coexistence of two morphs was allowed over a wide range of herbivory pressure and the cost of defence.
4. These results indicate that the optimal diet choice by *P. brassicae* contributes to the coexistence of hairy and glabrous *A. halleri*. While species interaction and stochastic dispersal are both involved in polymorphism dynamics in the field, our findings suggest that the role of consumer behaviours in the maintenance of defence polymorphism may be more important than currently appreciated.

**Keywords:** *Arabidopsis halleri* subsp. *gemmifera*; Associational effects; Genetic variation; Herbivory; Optimal diet choice; Plant defence

## INTRODUCTION

Many plant and animal species develop defence traits against their natural enemies. Genetic variation within species in defence traits has often been reported for both chemical and morphological traits (e.g. Blouw & Hagen 1984; Hauser, Harr & Schlötterer 2001; Hare & Elle 2004; Ruxton, Sherratt & Speed 2004; Wise, Yi & Abrahamson 2009; Moore *et al.* 2014). Polymorphism in defence levels within a prey or host species has been interpreted as a type of Batesian mimicry, the so-called “automimicry” (e.g. Brower 1960; Augner & Bernays 1998; Ruxton, Sherratt & Speed 2004; Svenningsen & Holen 2007; Moore *et al.* 2014). One potential mechanism maintaining dimorphism in defence levels is the negative frequency-dependent selection (also known as apostatic selection) (Sherratt & Harvey 1993; Ruxton, Sherratt & Speed 2004). If this type of selection occurs, the rarer morphs have higher fitness compared to the abundant ones and consequently increase in frequency over time, allowing multiple morphs to coexist within a population. Thus, evolutionary ecologists have often asked how negative frequency-dependent selection may be a consequence of consumer response to polymorphism in defence levels of resources (e.g. Matthews 1977; Ruxton, Sherratt & Speed 2004; Svenningsen & Holen 2007).

Optimal foraging behaviour of consumers has been proposed as a mechanism that can generate negative frequency-dependent selection (e.g. Murdoch 1969; Matthews 1977; Abrams 1982; Holt 1983). For example, the optimal diet choice (ODC) theory predicts that the density of preferred resource alters the optimal level of preference to suboptimal ones so that consumers maximize their energy gain (MacArthur & Pianka 1966; Charnov 1976). Because such a change in preference increases the consumption rate for suboptimal resource

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as the density of preferred resource reduces, the consumption following ODC potentially drives the negative frequency-dependent selection on resource polymorphism (Abrams 1982; Holt 1983). Indeed, several experimental studies have shown that such frequency-dependent consumption results in negative frequency-dependent selection between multiple morphs in animal prey (e.g. Brower 1960; Skelhorn & Rowe 2005; Fitzpatrick, Shook & Izally 2009) and consequently maintains the polymorphism in an experimental prey population (Bond & Kamil 1998). However, there is still limited evidence of the contribution of ODC to the maintenance of defence polymorphism in a natural population.

In plant–herbivore interactions, the risk of herbivory in individual plants depends not only on plants’ own phenotypes but also on those of the neighbouring plants (e.g. Barbosa *et al.* 2009; Wise, Yi & Abrahamson 2009; Sato & Kudoh 2016; Verschut *et al.* 2016). This phenomenon has been called “associational effects” (Barbosa *et al.* 2009; Underwood, Inouye & Hambäck 2014) and may influence plant’s coexistence through frequency-dependent herbivory (Underwood, Inouye & Hambäck 2014). To date, many empirical studies have suggested that herbivore behaviour mediates such associational effects between differentially defended plants (e.g. Hjältén, Danell, & Lundberg 1993; Bergvall & Leimar 2005; Rautio *et al.* 2012; Sato & Kudoh 2016; Verschut *et al.* 2016). For example, if herbivores choose among individual plants growing in a close proximity, this food preference could lead to an increased benefit of the defence, namely associational resistance, for unpalatable plants within patches (Bergvall & Leimar 2005; Sato & Kudoh 2016; Verschut *et al.* 2016; Hahn & Orrock 2016). If herbivores avoid unpalatable plants and prefer to palatable plants in a neighbourhood, associational susceptibility for palatable plants may occur at the same time within a patch (Vershut *et al.* 2016; Hahn & Orrock 2016). Theoretical studies also suggest that such a food choice by herbivores may lead to the associational effects and the coexistence of defended and undefended plants (Holt & Kotler 1987; Ishii & Crawley 2011;

Hambäck *et al.* 2014). Little is known, however, about the role of foraging behaviour of herbivores in associational effects and the maintenance of defence polymorphism.

Several plant species of the genus *Arabidopsis* exhibit dimorphism in trichome production within populations, having both trichome-producing (hereafter, hairy) and trichomeless (hereafter, glabrous) individuals (Hauser, Harr & Schlötterer 2001; Kawagoe *et al.* 2011). *Arabidopsis* trichomes confer resistance to leaf chewing herbivores (Mauricio 1998; Sletvold *et al.* 2010; Sato *et al.* 2014; Sato & Kudoh 2016) but impose a fitness cost on plants (Mauricio 1998; Sletvold *et al.* 2010; Kawagoe *et al.* 2011). In *A. halleri* (L.) O’Kane & Al-Shehbaz subsp. *gemmifera* (Matsum.) O’Kane & Al-Shehbaz [Brassicaceae], the brassica leaf beetle, *Phaedon brassicae* Baly [Coleoptera: Chrysomelidae], preferred glabrous plants when hairy ones were rare and thereby mediated a frequency-dependent damage between hairy and glabrous plants in the field (Sato *et al.* 2014). Our laboratory experiment also found that hairy plants incurred a growth cost of defence and the growth rate of each morph was negatively correlated with its own frequency under the defence–growth trade-off (Sato & Kudoh 2016). These findings suggest that the leaf beetle drives associational effects and consequently causes a frequency-dependent selection, but this hypothesis has not been tested explicitly.

In this study, we combined theoretical models and empirical data to test the hypothesis that foraging behaviours of *P. brassicae* contribute to the maintenance of trichome dimorphism in natural *A. halleri* populations. Specifically, the following three questions were addressed in this study: (1) Does optimal diet choice (ODC) account for the observed damage by leaf beetles in hairy and glabrous individuals? (2) Can ODC explain the observed patch dynamics of hairy and glabrous plants in the field? (3) Does ODC contribute to the coexistence of hairy and glabrous morphs via negative frequency-dependent selection?

## MATERIALS AND METHODS

### Study organisms

*Arabidopsis halleri* is a self-incompatible perennial herb distributed across Japan and the Russian Far East. Hairy individuals of this subspecies produce non-glandular trichomes on their leaf and stem surfaces (Fig. 1a), whereas glabrous plants have no trichomes except on leaf margins (Fig. 1b). The hairy and glabrous phenotypes are associated with allelic variations in a single gene *GLI* (Kawagoe *et al.* 2011) for which glabrousness is considered recessive. Plants have no specialized organs for long-distance seed dispersal and produce clonal rosettes after flowering (Fig. 1d). Our field survey was conducted in a natural *A. halleri* population in central Japan (Taka-cho, Hyogo; 35°06'N, 134°56'E; Sato *et al.* 2014). Thousands of *A. halleri* plants occur in fragmented habitats along a creek, and no Brassicaceae species other than the study species inhabits the field site. *Phaedon brassicae* is abundant in the flowering season and impacts plant fecundity by damaging the flowering stems (Kawagoe & Kudoh 2010).

*Phaedon brassicae* is a crucifer-feeding agricultural pest distributed across Asia (Ôtake & Funaki 1958; Wang *et al.* 2007). The beetle reaches maturity around 3 weeks after hatching (Wang *et al.* 2007). Adults are flightless and access their host plants by walking (Ôtake & Funaki 1958) (Fig. 1c), and larvae develop on individual plants on which they hatch (Ôtake & Funaki 1958). Thus, adults select their host plants not among but within plant patches, and the damage to individual plants is attributable to host choice by adults during oviposition.

## Leaf damage

We first focused on the foraging behaviour of the walking beetle *P. brassicae* in order to explain the pattern of leaf damage in hairy and glabrous plants. Individual *A. halleri* plants are spatially aggregated in the field; hereafter, this pattern of aggregation is denoted “patches”. The diameter of each plant patch was less than 1 m at the field site (Sato *et al.* 2014). At this patch scale, adult beetles walk freely among individual plants of *A. halleri* and feed on them. However, since *P. brassicae* are flightless, the neighbouring plant patches are far more distant (mean  $\pm$  SD =  $6.1 \pm 3.9$  m,  $n = 48$  patches) than the neighbouring plant individuals (mean  $\pm$  SD =  $9.0 \pm 5.7$  cm,  $n = 232$  plants). Besides, the plant density within a patch does not depend on the patch size as the average distance between neighbouring plants was not significantly correlated with the patch diameter (cm) ( $r = -0.17$ ,  $P = 0.25$ ,  $n = 46$  patches). These facts lead us to assume that interpatch movements are much more costly for *P. brassicae* than the intrapatch movements and rarely happen during their host choice; thus, we focused on the optimization of host plant choice by *P. brassicae* at individual level within patches.

We applied the optimal diet choice (ODC) theory (MacArthur & Pianka 1966; Charnov 1976) to model the behavioural response by which *P. brassicae* optimize their foraging depending on the number of hairy and glabrous plants within a patch. The parameters of this ODC model were estimated using the laboratory data on leaf damage in hairy and glabrous plants (Sato & Kudoh 2016) and implemented to predict the patterns of field data on leaf damage (Sato *et al.* 2014).

**Theoretical model.** We consider a plant population to be composed of spatially isolated patches in which hairy and glabrous plants may co-occur. Herbivores stroll among individual plants within a patch and optimize their foraging behaviour. Because the distance

between neighbouring *A. halleri* plants is independent of the patch size, we assume that the herbivore encounters any plant individual within the patch at a constant rate  $P$ . Thus, the encounter rates for glabrous and hairy plants are represented as  $PN_g/(N_g+N_h)$  and  $PN_h/(N_g+N_h)$ , respectively, where  $N_g$  denotes the number of glabrous and  $N_h$  the number of hairy plants within the patch. We represent the handling time of glabrous and hairy plants for herbivores as  $H_g$  and  $H_h$  and their energy intake as  $E_g$  and  $E_h$ , respectively. We assumed that for herbivores glabrous plants are a more profitable resource compared to hairy plants, i.e.  $E_g/H_g > E_h/H_h$ . According to the ODC model (Charnov 1976), feeding on both glabrous and hairy plants is optimal for herbivores when

$$\frac{E_h}{H_h} > \frac{\frac{PN_g}{N_h + N_g} E_g}{1 + \frac{PN_g}{N_h + N_g} H_g}. \quad \text{Eq. (1)}$$

Otherwise, feeding only on glabrous plants gives herbivores better energy acquisition than feeding on both. Solving Eq. (1) by using the frequency of hairy plants  $f = N_h/(N_h+N_g)$ , we obtain the threshold frequency of hairy plants at which herbivores should switch their foraging tactics  $f^*$ , i.e.

$$f^* = 1 - \frac{se}{h-e}, \quad \text{Eq. (2)}$$

where  $e = E_h/E_g$ ,  $h = H_h/H_g$ , and  $s = 1/PH_g$ .

Although the original ODC theory predicts the abrupt change of preferences to a suboptimal resource at  $f = f^*$ , in nature such a preference will gradually change with the frequency of the suboptimal resource (Stephens & Krebs 1986) because of the incorrect estimation of the frequency of suboptimal plant or the limited knowledge of the environment (McNamara & Houston 1987; Křivan 2010). Thus, we assumed that the probability of feeding on hairy plants  $D(f)$  gradually changes with the frequency of hairy plants  $f$ . Following



the formula of McNamara and Houston (1987),

$$D(f) = \frac{1}{1 + \exp[-\alpha(f - f^*)]}, \quad \text{Eq. (3)}$$

where  $\alpha$  denotes the accuracy of herbivore response; at very large  $\alpha$  the  $D(f)$  values are the same as those predicted by the original ODC model (i.e.  $D(f) = 0$  when  $f < f^*$ ,  $D(f) = 1$  when  $f \geq f^*$ ). Note that the probability of feeding on glabrous plants is always one, because the partial preference for glabrous plants always reduces the total energy acquisition.

When herbivores feed on plants as the result of partial preference to hairy plants  $D(f)$ , the damage by herbivory to glabrous and hairy plants are

$$\frac{\varepsilon}{s + (1 - f) + fhD(f)} \frac{M(N_g, N_h)}{N_g + N_h} \quad \text{and} \quad \text{Eq. (4a)}$$

$$\frac{\varepsilon D(f)}{s + (1 - f) + fhD(f)} \frac{M(N_g, N_h)}{N_g + N_h}, \text{ respectively,} \quad \text{Eq. (4b)}$$

where  $\varepsilon$  is the coefficient of damage per attack and  $M(N_g, N_h)$  is the number of herbivores within the patch (see Appendix S1 in Supporting Information for the derivation). Although the functional shape of  $M(N_g, N_h)$  has often been discussed (cf. Hambäck *et al.* 2014), the damage to *A. halleri* was proportional to the number of plants in a patch in our study site (Appendix S2); therefore, in the following analysis we assumed the linear relationship, i.e.  $M(N_g, N_h) = m(N_g + N_h)$  (but see Appendix S2 for non-linear analyses). Then, the damages to a glabrous and hairy plant are

$$\frac{v}{s + (1 - f) + fhD(f)} \quad \text{and} \quad \text{Eq. (5a)}$$

$$\frac{vD(f)}{s + (1 - f) + fhD(f)}, \text{ respectively,} \quad \text{Eq. (5b)}$$

where  $v = \varepsilon m$ .

**Data.** Two datasets were used to predict herbivory by *P. brassicae*. The laboratory experimental data provided the extent of leaf damages (evaluated as a proportion of lost leaf area) by adult *P. brassicae* under two ratios of hairy to glabrous individuals (3:1 and 1:3) (Sato & Kudoh 2016). The field observational data provided the extent of leaf damage (evaluated as the proportion of leaf area lost to herbivory; scored from 0 to 1.0 at 0.1 increments) in hairy and glabrous plants in a set of circular monitoring plots (1 m in diameter) (Sato *et al.* 2014). The details of the field surveys and experiments are described in previous publications (Sato *et al.* 2014; Sato & Kudoh 2016). The dataset used in the present study is available at the Dryad (doi: XXXXX).

**Statistical analysis.** We first estimated the model parameters of ODC ( $e$ ,  $h$ ,  $s$ , and  $\alpha$ ) from the laboratory experimental data on leaf damage (Sato & Kudoh 2016). The leaf damage to individual plants was fitted by a least-square method. For the parameter estimation, we used the ‘optim’ function implemented in R version 3.1.0 (R Core Team 2015). The initial parameters were set at  $e = h = s = \alpha = 1.0$ . We first applied the Nelder-Mead method to avoid local optima, and then used the quasi-Newton method (BFGS method in R) to calibrate the parameters. Biases in these parameters were estimated using 95% percentiles of 10,000 bootstrap samplings with replacement of the leaf damage data in individual plants.

To validate the parameters estimated from the laboratory data, we then compared the patterns of leaf damage in hairy and glabrous plants between the model prediction and field data (Sato *et al.* 2014). Given that the herbivore abundance and spatial scales differed between the field and laboratory, we scaled the model prediction to the level of field damage as: Field damage =  $\beta \times$  Damage predicted by the ODC model (using the ‘nls’ function in R).

## Plant demography

Because of the impact of herbivory on plant fecundity and/or mortality, foraging behaviour of *P. brassicae* may affect the demographic dynamics of plants via negative frequency-dependent selection between hairy and glabrous plants. The demography of herbivores in each patch may be translated into herbivory pressure, which in turn influences plant demography. For example, a better energy acquisition will enhance population growth of herbivores, resulting in higher herbivory pressure in the next year. However, our field data showed that the past leaf damage had a negligible influence on the current leaf damage (Appendix S2), and therefore, we focused on the demography of hairy and glabrous *A. halleri* and ignored the population dynamics of *P. brassicae*.

We developed a plant demography model where the plant mortality and/or fecundity were affected by the damage derived from the ODC model, Eq. (5a) and (5b). The parameters of the plant demography model were estimated by fitting the model to the field data on the annual change in the number of hairy and glabrous plants in a set of patches. We then used the estimated parameters to test how ODC by *P. brassicae* contributes to the coexistence of hairy and glabrous plants in natural *A. halleri* populations.

**Theoretical model.** We considered that the field demography processes of plants were characterized by two sequential events: death and recruitment. In the death event, some of the plants die and thereby the number of plants in a patch decreases. We assumed that the mortality of glabrous and hairy plants increased linearly with the damage caused by herbivory, i.e.

$$m_c + m_d \frac{v}{s + (1-f) + fhD(f)} \quad \text{and} \quad \text{Eq. (6a)}$$

$$m_c + m_d \frac{vD(f)}{s + (1-f) + fhD(f)}, \text{ respectively,} \quad \text{Eq. (6b)}$$

where  $m_c$  is the constant intrinsic mortality, and  $m_d$  is the coefficient of additional mortality due to the damage by herbivory. The total number of dead plants is represented as a binominal distribution with the probability given by Eq. (6a) and (6b) (see Appendix S3 for details).

In the recruitment event, new plants are recruited in each patch and thereby the number of plants in the patch increases. We assumed that the fecundity (i.e. the number of seeds or clones) linearly decreased with damage (Eq. (5a) and (5b)) and the cost of trichome production  $c$ . Then, the total fecundity of glabrous and hairy plants in a focal patch are

$$R \left[ 1 - \frac{v}{s + (1-f) + fhD(f)} \right] n_g \quad \text{and} \quad \text{Eq. (7a)}$$

$$R \left[ 1 - \frac{vD(f)}{s + (1-f) + fhD(f)} - c \right] n_h, \text{ respectively,} \quad \text{Eq. (7b)}$$

where  $R$  is the potential number of offspring per capita, and  $n_g$  and  $n_h$  are the number of glabrous and hairy plants within the patch, respectively. In *A. halleri*, the plants produce clonal rosettes and seeds are dispersed by gravity. Thus, we assumed that a fraction of produced offspring is dropped within the same patch, while the others reach different patches that are randomly selected from the population. When we represent the former and the latter fraction as  $1-d$  and  $d$ , respectively, the number of offspring of glabrous and hairy plants reaching a focal patch are

$$d\tilde{R}_g + (1-d)R \left[ 1 - \frac{v}{s + (1-f) + fhD(f)} \right] n_g \quad \text{and} \quad \text{Eq. (8a)}$$

$$d\tilde{R}_h + (1-d)R \left[ 1 - \frac{vD(f)}{s + (1-f) + fhD(f)} - c \right] n_h, \text{ respectively, Eq. (8b)}$$

where  $\tilde{R}_g$  is the mean value of Eq. (7a) and  $\tilde{R}_h$  is the mean value of Eq. (7b) in the population.

We assumed that all the offspring that reached the focal patch will establish and mature in that patch (However, our results were unchanged even when we incorporated the effect of

patch size on the invasion difficulty; see Appendix S4.). Although the distribution of the number of newly established plants is uncertain, we approximate the probability distribution of the number of established individuals by a Poisson distribution with the mean given by Eq. (8a) and (8b).

In *A. halleri*, individual plants reproduce once a year in spring (Kawagoe & Kudoh 2010), while the death event occurs throughout the year. Our field observations were conducted at the end of reproductive season of each year (mid-May; see the next subsection). Thus, the annual life cycle of *A. halleri* can be represented by a single recruitment event followed by a single death event. Consequently, we can quantify the occurrence probability of annual demographic changes by summing up the probability of all possible combinations for the number of dead and recruited plants, which follow a binominal distribution (with the probability as defined by Eq. (6a) and (6b)) and a Poisson distribution (with the mean defined by Eq. (8a) and (8b)), respectively (see Appendix S3 for details of the derivation).

**Data.** Once a year from 2013 to 2016, we randomly selected 80 plant patches and set circular monitoring plots to count the number of all hairy and glabrous plants in the plots. Given that the number of plants within the circular area approached a plateau at 1-m diameter in this site (Sato *et al.* 2014), the diameter of circular plots was set at 1 m to monitor patch size dynamics. The survey was conducted in the late flowering season (mid-May). Leaf damages during this study period fully reflected the level of herbivory on individual plants as it occurred immediately after the spring outbreak of *P. brassicae*. We surveyed ca. 80 plots per year and obtained 243 measurements of their yearly dynamics. Given the clonality of *A. halleri*, a plant with no vegetative connection with others was designated as an individual in this survey.

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Additionally, we used laboratory data on the biomass (mg) of hairy and glabrous plants to compare the estimated cost of defence  $c$  with our previous evidence on the growth cost of trichomes (Sato & Kudoh 2016). Potted plants were cultivated for 6 months in an indoor space without herbivory. The intrinsic growth cost for hairy plants was calculated as  $[\ln(\text{median glabrous biomass}) - \ln(\text{median hairy biomass})] / \ln(\text{median glabrous biomass})$ . The details regarding the cultivation are described in Sato and Kudoh (2016). The dataset used in the present study is available at the Dryad (doi: XXXXX).

**Statistical analysis.** Using the estimated parameters of the ODC model ( $e$ ,  $h$ ,  $s$ , and  $\alpha$ ), we then estimated the six parameters ( $v$ ,  $R$ ,  $c$ ,  $d$ ,  $m_c$ , and  $m_d$ ) of the plant demography model. The field patch data on the number of hairy and glabrous plants observed from 2013 to 2015 were used to quantify the likelihood of the observed field dataset pertaining to annual patch dynamics in our plant demography model (see Appendix S3 for derivation). To increase the log-likelihood of observed data, we ran eight independent chains of Markov chain Monte Carlo (MCMC) with  $10^6$  iterations,  $10^3$  thinning interval, and  $10^5$  burn-in period, starting from the following initial parameters:  $v = 0.0$ ,  $R = 1.0$ ,  $c = 0.0$ ,  $d = 0.5$ ,  $m_c = 0.5$ , and  $m_d = 0.01$ . The mortality due to herbivory  $m_d$  was estimated at an exponential scale to examine a range of positive values. Proposal parameters were generated following a normal distribution  $N(\mu_t, \sigma^2)$ , where  $\mu_t$  denotes the parameter value at the current iteration, and  $\sigma$  is set at 0.25 for  $v$  and  $R$  and at 0.025 for  $c$ ,  $d$ ,  $m_c$ , and  $m_d$ . These proposals were accepted/rejected following the Metropolis algorithm (Hastings 1970). The convergence of MCMCs was assessed by  $\hat{R}$  statistic at  $< 1.1$  (Plummer *et al.* 2006). If parameters were estimated to nearly zero, the  $\hat{R}$  statistic was calculated after excluding such ineffective parameters. The present modelling and parameter estimation were conducted using the Rcpp (Dirk & Francois 2011), BH (Dirk, Emerson & Kane 2015), coda (Plummer *et al.* 2006) package, and Rtools version

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3.3.0.1958 (available from: <https://cran.r-project.org/bin/windows/Rtools/>). (The R code is available at the Dryad, doi: XXX)

## RESULTS

### Prediction of leaf damage patterns

We first estimated the parameters of our ODC model by fitting the predicted damage, Eq. (5a) and (5b), to the laboratory data. Glabrous plants were a more profitable resource for *P. brassicae* than hairy plants as shown by the greater handling time ( $h = 1.82$ ; Table 1a) and lower energy intake ( $e = 0.58$ ; Table 1a) of hairy plants. Given the set of the estimated handling time, energy intake, searching time, and response accuracy in the laboratory (Table 1a), our ODC model predicted that *P. brassicae* avoided hairy plants when hairy plants were rare, but fed on both hairy and glabrous plants when hairy ones were abundant (solid lines and circles in Fig. 2a, b). In other words, the defensive role of producing hairs is effective when the frequency of glabrous plants is increased, whereas glabrous plants receive weak effects from neighbours. This result shows that the ODC by *P. brassicae* causes the associational effects.

Our ODC model with the estimated parameters (Table 1a) provided a comparable pattern with the observed trends in the field, as the intervals of prediction overlapped between the model (solid lines in Fig. 2a, b) and field data (dashed lines in Fig. 2a, b) on leaf damages in the two morphs. Hairy plants were less damaged when they were rare, whereas the damage to glabrous plants did not depend on the frequency of the two morphs in the field (dashed lines and pale plots in Fig. 2a, b). These results indicate that the ODC by *P. brassicae* accounts for the pattern of leaf damage to hairy and glabrous plants.

## Prediction of plant demography

Based on the estimated parameters of the ODC model (Table 1a), we next examined the sets of six parameters of the plant demography model that increase the likelihood of observed field data (Table 1b). The herbivory pressure  $v$ , cost of defence  $c$ , interpatch dispersal  $d$ , and intrinsic mortality  $m_c$  were involved in the field demography of hairy and glabrous plants, whereas the mortality due to herbivory  $m_d$  was negligible at nearly zero (Table 1b). The growth cost for hairy plants directly compiled from the laboratory biomass data showed a smaller but comparable level of the defence cost (empirical  $c = 0.09$  [0.02–0.13], bootstrap median with 95% confidence interval [CI]) with the estimated cost  $c$  ( $c = 0.22$  [0.15–0.24], median with 95% CI; Table 1b). Approximately 10% of the recruitment process was attributable to the interpatch dispersal ( $d = 0.10$  [0.08–0.13]; Table 1b). Convergence of MCMCs was confirmed by  $\hat{R}$  statistic ( $\hat{R} = 1.015$ ).

Then, we tested the validation of our estimation by forecasting the test data. Using the parameters estimated from the data obtained in the first three years (2013 to 2015), we numerically simulated plant demography for 2015 to 2016 and then compared the predicted dynamics with the morph-frequency and patch size observed in 2016. The probability density of the frequency of hairy plants in a patch was comparable with that observed in 2016 (Fig. 2c). The number of *A. halleri* plants observed in 2016 ranged within those predicted by the model (Fig. 2d). Although we assumed ODC by *P. brassicae*, the observed demography might be predicted without ODC. Therefore, we estimated the model parameters assuming the absence of ODC, i.e. no difference between glabrous and hairy plants for herbivores ( $h = e = 1$ ). This model selection showed that the model without ODC was less fitted to the observed demography than that with ODC (difference of the deviance information criterion,  $\Delta\text{DIC} = 9.95$ ; see also Appendix S5). These results indicate that the morph-frequency and patch size dynamics are predictable by the plant demography model incorporating ODC.



We further assessed whether the estimated model showed a similar morph-frequency as observed in the natural population. The frequency of hairy plants at the equilibrium state was derived by analysing the condition under which fitness of the two morphs becomes equal (by solving Eq. (7a) and (7b); see Appendix S6 for derivation). We analysed the equilibrium frequency and its confidence intervals using the parameter sets sampled from MCMCs. These parameter sets predicted the intermediate frequency of hairy plants (predicted  $f = 0.53$  [0.45–0.62], median [95% CI]), and this interval of predicted frequency was overlapped with the observed frequency of hairy plants in the natural population (observed  $f = 0.52$  [0.47–0.58] estimated by 10,000 bootstrap replicates on plant patches surveyed in 2016). Thus, the estimated model provides quantitative prediction of the observed frequency in natural population.

### **Coexistence of hairy and glabrous plants**

To test whether the model with estimated parameters (Table 1) predicts a negative frequency-dependent selection, we asked (1) whether rarer morphs have higher fitness than abundant ones and (2) whether frequencies of rarer morphs increase over time. First, we calculated the relative fitness of hairy and glabrous plants with respect to the frequency of hairy plants in the population (Fig. 3a). The model predicts that relative fitness of one morph increases as it becomes relatively rare (Fig. 3a), showing negative frequency-dependent selection between the two morphs. Second, because the result based on the relative fitness does not include the effect of patch structure at the population scale, we performed numerical simulations to confirm negative frequency-dependent selection at such a population scale. We constructed a virtual plant population whose patch size followed the Poisson distribution with a mean patch size observed in the natural population ( $N_g + N_h = 10.03$ ) and simulated the patch size dynamics by applying the model with the estimated parameters (Table 1). The

simulated dynamics showed that the frequency of hairy plants at the population scale approached the equilibrium frequency over time (blue points in Fig. 3b) even when the meta-patch structure was taken into consideration. Contrarily, a numerical simulation without any effects of ODC (i.e.  $h = e = 1$ ) did not show the frequency-dependent selection between the two morphs (red points in Fig. 3b). These results indicate that ODC by leaf beetles promotes the maintenance of trichome dimorphism via negative frequency-dependent selection.

Finally, we analysed the conditions under which the herbivory pressure  $v$  and defence cost  $c$  allow hairy and glabrous plants to coexist within a population (Fig. 3c; see also Appendix S6 for derivation). The estimated range of herbivory pressure  $v$  and the defence cost  $c$  was included within the predicted region where hairy and glabrous plants coexist (Fig. 3c). This region for coexistence was broader than that for extinction of one morph. This analysis indicates that, in the presence of ODC by leaf beetles, hairy and glabrous plants likely coexist even if the herbivory pressure and cost of defence vary across a wide range.

## DISCUSSION

### **Optimal forager promotes the maintenance of polymorphism**

How natural enemies govern prey diversity is an intriguing and long-standing question in trophic interactions (e.g. Brower 1960; Murdoch 1969; Ruxton, Sherratt & Speed 2004).

Although several studies to date have shown the importance of natural enemies for coexistence of multiple prey (or host) species (Ishii & Shimada 2012) or morphs (Bond & Kamil 1998) in the laboratory, quantitative evidence in natural populations remains limited. Here, we have shown that optimal diet choice (ODC) by herbivores is a crucial mechanism stabilizing the coexistence of hairy and glabrous plants in a natural population based on the

following three results. First, the pattern of leaf damage to hairy and glabrous plants was explained by ODC by the leaf beetles. Second, the model incorporating ODC better predicted plant demography than the model without ODC. Third, the numerical analysis and simulation revealed that ODC by the leaf beetle has driven a negative frequency-dependent selection that maintains the dimorphism of hairy and glabrous plants. These results present one of few field examples that demonstrate the stabilizing role of natural enemies in defence polymorphism.

The prediction of ODC wherein consumers feed on suboptimal resource when preferred resource is rare has been supported for many species (Pyke, Pulliam & Charnov 1977; Sih & Christensen 2001), especially those feeding on sessile prey (Sih & Christensen 2001). This prediction is applicable to adult host choice and consequent larval performance in herbivorous insects (Jaenike 1978; Scheirs & Bruyn 2002). Hairy plants are considered suboptimal for *P. brassicae* since larvae grow better when they are fed glabrous leaves than hairy leaves (Sato *et al.* 2014). Given that larvae of *P. brassicae* have no opportunity to actively choose host plants (Ôtake & Funaki 1958), plant damages are attributable to adult host choice followed by larval feeding. Although the proximate mechanisms by which adult beetles recognise hairy and glabrous plants have yet to be elucidated, ODC is a plausible mechanism underlying the pattern of plant damage.

Qualitatively speaking, the maintenance of defence polymorphism under herbivore-mediated interactions has often been discussed in the context of game theory that assumes the intrinsic cost and associational benefits of the defence among defended and undefended plants (Augner & Bernays 1998; Rautio *et al.* 2012). There is a growing body of evidence on the cost of physical and chemical defence against herbivores (e.g. Mauricio 1998; Hare & Elle 2004; Sletvold *et al.* 2010). More importantly, recent studies on associational effects have shown that food preference by herbivores causes associational resistance for unpalatable plants and simultaneously leads to associational susceptibility for

plants within a patch (Verschut *et al.* 2016; Hahn & Orrock 2016). In contrast, ODC by the leaf beetle *P. brassicae* predicts intrapatch associational resistance for hairy plants, but not associational susceptibility for glabrous plants. This is because the handling time on hairy plants is much longer than that on glabrous plants ( $h = 1.82$ ; Table 1a) and thereby the existence of hairy plants decreases the attack rate to glabrous plants. As discussed in the former paragraph, optimal foragers are expected to feed on unpalatable food only when palatable food becomes rare (MacArthur & Pianka 1966; Charnov 1976). In terms of ODC, our present study explains why only the associational resistance for unpalatable plants occur within a patch.

Remarkably, the coexistence of two morphs was widely predicted across the parameter region of the defence cost  $c$  or herbivory pressure  $h$ . For example, under the estimated parameters, the coexistence can still be possible even if the defence reduces the reproductive success by almost 50% (Fig. 3c). Moreover, according to the estimated parameters (Table 1a), the preference for hairy plants changes very gradually with the frequency of the hairy plants, i.e. the probability of feeding on hairy plants  $D(f)$  is expected to be 0.43 and 0.90 when the patch is filled by glabrous plant ( $f = 0$ ) and hairy plant ( $f = 1$ ), respectively. Therefore, neither the subtle balance between cost and benefit of the defence nor the high accuracy of herbivore foraging is a necessary condition for the coexistence of two morphs. This line of analysis implies that the stabilizing role of ODC by herbivores in the maintenance of defence polymorphism may be widespread in plant–herbivore and other trophic interactions.

## Disentangling species interaction under complex field environment

Spatial heterogeneity within a single population is hypothesized to be important as plants are incapable of escaping from herbivores (Ishii & Crawley 2011; Hambäck *et al.* 2014).

Considering a meta-patch structure and the intrapatch effects of herbivore foraging, we were able to evaluate the relative importance of interpatch dispersal and intrapatch herbivory in the morph-frequency dynamics. The result that showed that the intrapatch dispersal of plants was predominant compared to the interpatch dispersal (as indicated by the estimated proportion of interpatch dispersal,  $d = 0.10$ ) seems convincing, because *A. halleri* plants disperse their offspring near a maternal plant by clonal propagation or seed dispersal by gravity. Separating the plant life cycle into the two stages, we also uncovered the impact of herbivory on the recruitment but not on the death stage. This result agrees with the fact that *P. brassicae* become abundant in the recruitment season, decreasing plant fecundity (Kawagoe & Kudoh 2010). Given that *A. halleri* individuals are rarely killed by infestation by *P. brassicae* alone (only one dead out of 120 plants as reported by Sato & Kudoh 2016), it was conceivable that herbivory did not affect mortality.

Although our study found that foraging behaviour of *P. brassicae* contributes to the maintenance of polymorphism in *A. halleri*, this does not necessarily indicate the absence of other factors affecting the frequency of defended plants within a population. Co-occurrence of defended and undefended morphs might also be allowed by spatiotemporal variation in herbivory pressure and migration among populations (Hare & Elle 2004; Andrew *et al.* 2007; Sato & Kudoh 2017). The frequencies of hairy and glabrous plants and the prevalent herbivore species indeed vary among *A. halleri* populations (Sato & Kudoh 2015; Sato & Kudoh 2017). Notably, the present modelling approach enables us to quantify the stability of the morph frequency caused by ODC (Fig. 3b) or to perform a model selection that compares the predictability among multiple models (e.g. DICs). Although temporal dynamics between

the current and past herbivory pressure were unclear in our 4-year survey (Appendix S2), this effect might be significant in long-term data. Further understanding of the relative importance of multiple factors responsible for the co-occurrence of defended and undefended morphs will be possible by analysing the extended model combined with the long-term demography data from multiple plant populations.

## Conclusion

Our present findings suggest that adaptive foraging by natural enemies may play a more important role in stabilizing defence polymorphism in the wild than previously thought. Recent studies have begun to adopt a likelihood approach to detect the ongoing frequency-dependent selection from the time-series polymorphism dynamics (Rouzic *et al.* 2015) or to test the mechanisms underlying the coexistence of plant genotypes (Miller & Rudgers 2014). Although our present study focused on *A. halleri* and *P. brassicae*, this type of modelling may be applied to other systems. Several studies on plant–herbivore interaction suggest that scales of associational effects depend on spatial structure of herbivores and plants (Hjältén, Danell, & Lundberg 1993; Sato & Kudoh 2015; Verschut *et al.* 2016). Now that evidence for apparent interaction has been accumulated in various trophic interactions (see Ruxton, Sherratt & Speed 2004 for animals; Barbosa *et al.* 2009 for plants), joint approaches using optimality modelling and empirical data will enable us to address how behavioural basis of natural enemies impacts prey diversity across ecosystems.

## AUTHOR CONTRIBUTIONS

YS collected and analysed the data. KI developed the mathematical models. YS, KI, and HK conceived the study and wrote the paper.

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## DATA ACCESSIBILITY

Source codes and data are deposited in the Dryad Digital Repository:

<http://dx.doi.org/10.5061/dryad.pn088> (Sato, Ito & Kudoh 2017)

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1 Derivation of the damage

Appendix S2 Total amount of herbivory

Appendix S3 Derivation of the likelihood

Appendix S4 Influence of patch size

Appendix S5 The importance of ODC

Appendix S6 Condition for the coexistence

**Table 1** Estimated parameters of the herbivore behaviour and plant demography model.

(a) Least-square estimates for four parameters responsible for leaf damage to hairy and glabrous plants in the laboratory. The optima estimated by the Nelder-Mead and quasi-Newton method are shown with bootstrap confidence intervals (CIs).

Parameter	Description	Estimate	Bootstrap 95% CI	
			Lower	Upper
$h$	Handling time	1.81	1.39	2.58
$e$	Energy intake	0.58	0.48	1.28
$s$	Searching time	1.88	1.56	2.34
$\alpha$	Response accuracy	2.52	1.58	3.05

(b) Markov Chain Monte Carlo estimates for six parameters determining plant demography in the field. The median and 95% lower and upper percentiles are shown for parameter distributions.

Parameter	Description	Percentiles		
		Median	2.5%	97.5%
$v$	Herbivory pressure	2.57	2.07	2.72
$R$	Intrinsic growth rate	12.87	5.61	21.84
$c$	Cost of defence	0.22	0.15	0.24
$d$	Proportion of interpatch dispersal	0.10	0.08	0.13
$m_c$	Intrinsic mortality	0.75	0.69	0.80
$m_d$	Mortality due to herbivory	$<10^{-8}$	$<10^{-36}$	0.03
---	<i>Log-Likelihood</i>	-730.3	-735.2	-728.4

## Figure legends

**Figure 1** Photographs of *Arabidopsis halleri* subsp. *gemmifera* and *Phaedon brassicae*: (a) hairy plant; (b) glabrous plant; (c) adult beetle; and (d) clonal rosette producing roots.

**Figure 2** Prediction of leaf damage patterns and demography of hairy and glabrous plants.

Leaf damage (evaluated by proportion of the lost leaf area) to hairy (a) or glabrous (b) plants predicted by the optimal diet choice (left axis) and observed in the field (right axis). Solid curves and circles indicate the prediction ( $\pm 95\%$  CI) and average damage ( $\pm$ SE) in the laboratory (lab.), respectively. Dashed curves and pale circles show linear prediction ( $\pm 95\%$  CI) and average damage in the field. Prediction of the morph-frequency (c) and patch size (d) dynamics observed from 2015 to 2016. (c) The plot size corresponds to the number of plants in a patch observed in 2015. The darkness of the squares represents the probability density of the simulated frequency changes. (d) Dashed lines indicate the median and 95% prediction intervals of the model simulation.

**Figure 3** Negative frequency-dependent selection and the coexistence of hairy and glabrous plants as shown by the estimated parameters. (a) Relative fitness between hairy (solid line) and glabrous (dashed line) plants based on Eq. (S9a) and (S9b) in Appendix S6. (b) Population level dynamics of the morph-frequency simulated by models with (blue plots; solid line) or without (red plots; dashed line) the optimal diet choice (ODC). Trend lines are added using a smooth spline method. A single point indicates a simulated plant population consisting of 1,000 patches, and the simulation was repeated 100 times for each frequency. (c) Condition for the coexistence of hairy and glabrous plants under combinations of the herbivory pressure  $v$  and defence cost  $c$ . Circles and bars indicate the median and 95% percentiles of the estimated  $v$  and  $c$ .



