

LETTER

Herbivore community promotes trait evolution in a leaf beetle via induced plant response

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Abstract

Several recent studies have emphasised that community composition alters species trait evolution. Here, we demonstrate that differences in composition of local herbivore communities lead to divergent trait evolution of the leaf beetle *Plagioderma versicolora* through plant-mediated indirect interactions. Our field surveys, genetic analyses and community-manipulation experiments show that herbivore community composition determines the degree of herbivore-induced regrowth of willows (Salicaceae), which in turn, promotes the divergent evolution of feeding preference in the leaf beetle from exclusive preference for new leaves to a lack of preference among leaf-age types. Regrowth intensity depends both on the differential response of willows to different herbivore species and the integration of those herbivore species in the community. Because herbivore-induced regrowth involves phenological changes in new leaf production, leaf beetle populations develop divergent feeding preferences according to local regrowth intensity. Therefore, herbivore community composition shapes the selection regime for leaf beetle evolution through trait-mediated indirect interactions.

Keywords

Biodiversity, diffuse selection, eco–evolutionary dynamics, herbivore-induced plant response, local adaptation, niche construction, phenotypic plasticity, trait-mediated indirect effect.

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INTRODUCTION

Biologists have recognised that species interactions can affect species evolution since Darwin (1859). However, evolutionary studies have long focused on pairwise interactions between two species, such as predation, competition and mutualism. Only recently have several studies emphasised the role of community composition in altering species trait evolution (Rundle *et al.* 2003; Thompson 2005; Haloin & Strauss 2008; Lankau & Strauss 2008; Post & Palkovacs 2009; terHorst 2010).

Most studies on evolution in a community context have been concerned with direct trophic interactions and food webs (Benkman *et al.* 2001; Stinchcombe & Rausher 2002; Rundle *et al.* 2003; Palkovacs & Post 2008; terHorst 2010). This is likely because trophic interactions are traditionally considered to be one of the primary mechanisms that govern the number of interacting organisms, and thus shape ecological communities (Polis & Winemiller 1996). On the other hand, ecologists have begun to recognise that trait-mediated indirect effects (i.e. when a species shows a phenotypic change in response to an interaction with another species, the trait change influences subsequent interactions with third species) also play an important role in structuring ecological communities (Werner & Peacor 2003; Ohgushi 2005; Preisser *et al.* 2005). Although empirical evidence is limited, trait-mediated indirect effects may be a causal mechanism that imposes diffuse selection (i.e. selection which depends on the presence or absence of other species in a community; Inouye & Stinchcombe 2001; Strauss *et al.* 2005). Thus, it is necessary to incorporate trait-mediated indirect interactions into an evolutionary framework to

bridge the gap between community ecology and evolutionary biology.

In herbivore insect communities, species commonly interact through herbivore-induced plant responses: prior damage by one herbivore species triggers a plant phenotypic change that influences growth, survival and reproduction of another herbivore species sharing the same host plant (Denno *et al.* 1995; Karban & Baldwin 1997; Ohgushi 2005). Induced plant responses are ubiquitous across terrestrial plants, and provide a mechanistic basis for trait-mediated indirect interactions (Denno *et al.* 1995; Karban & Baldwin 1997; Ohgushi 2005; Kaplan & Denno 2007; Utsumi *et al.* 2010). A single plant species can exhibit diverse responses to attack by a wide range of herbivore species (Agrawal 2000; Van Zandt & Agrawal 2004; Kessler & Halitschke 2007; Rasmann & Turlings 2007), and simultaneous attack by multiple herbivore species often triggers differential plant responses than would result from a single-species attack (Voelckel & Baldwin 2004; Rasmann & Turlings 2007; Rodriguez-Saona *et al.* 2010). Consequently, community composition of herbivorous insects may alter the phenotypic expression of host plants. Furthermore, herbivorous insects may locally adapt to altered plant phenotypes, because insects possess far shorter lifespans than their long-lived host plant species. In fact, short lifespans with restricted gene flow of herbivorous insects often result in adaptations to individual host plants (Edmunds & Alstad 1978; Tack & Roslin 2010).

Willows (Salicaceae) express new lateral shoot production as a regrowth response following attacks by several herbivorous insect taxa (Nakamura *et al.* 2003; Utsumi & Ohgushi 2007; see Fig. S1). Willow regrowth response influences the phenology of leaf

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production, expanding a period of new leaf production due to vigorous lateral shoot growth (Nakamura *et al.* 2003; Utsumi *et al.* 2009). Utsumi *et al.* (2009) reported that willow regrowth have a potential to act as a selective force on feeding preference of the willow leaf beetle *Plagiodera versicolora* (Chrysomelidae), which feeds exclusively on willow leaves and emerges continuously from spring to autumn due to multiple generations. Where new leaves are seasonally abundant due to strong willow regrowth, leaf beetle populations develop exclusive preference for new leaves. But, other populations show a lack of preference (non-preference) among leaf-age types where new leaves are scarce and mature leaves are available due to weak willow regrowth (Utsumi *et al.* 2009). If induced willow regrowth depends on herbivore species identity in a community, herbivore community composition may alter the direction and magnitude of selection for the leaf beetle through induced plant response.

In this study, we hypothesised that differences in local insect community composition would alter the selective force on the leaf beetle *P. versicolora*, resulting in among-population divergence in beetle feeding preference. We specifically test how herbivore community structure determines willow regrowth expression, and how differences in the regrowth intensity subsequently lead to evolutionary divergence in *P. versicolora* preferences.

MATERIAL AND METHODS

Herbivore community and plant responses

The effects of herbivore community structure on willow regrowth were examined by conducting a field survey at eight sites in Japan and nine sites in Finland, each within 460-km range respectively (see Fig. S2). *Salix eriocarpa* and *S. caprea* (Salicaceae) were the respective dominant willows in Japan and Finland sites. Study sites were randomly selected in both countries.

In 2008, 10 trees with an average of 15-cm basal diameter were randomly selected at each of eight sites in Japan. Ten 1-year shoots were randomly selected from each tree. One half of the shoots were assigned to herbivore removal, and the remainder exposed to natural herbivory. In early May, each shoot assigned to herbivore removal was covered with high light transmissibility 1-mm polyethylene net mesh (Nihon Widecloth Co., Ltd., Osaka, Japan). We monthly recorded the number of newly emerged lateral shoots as regrowth response. Because new lateral shoots emerged only when plants were exposed to herbivory (see Fig. S1), the mean number of regrowth shoots per current-year shoot in July was used as an index of regrowth intensity. In mid-June, we collected insects from each tree by both manual sampling and beating with a silk net. All insects were immediately placed in plastic bags in a cool box, and were transported to a laboratory. We identified all herbivore species and recorded the number of each species in the laboratory.

Willow regrowth ability was examined by conducting a greenhouse experiment at the Center for Ecological Research (CER), Kyoto University. In April 2008, four willow cuttings were collected from each of all the study trees in the field census, and were grown in plastic pots with compost under greenhouse conditions. In early October 2008, we placed a second-instar *Clostera anastomosis* (Notodontidae: Lepidoptera) caterpillar on one half of the experimental cuttings from each study tree. *Clostera anastomosis* is a common herbivore on willows and poplars. The other half of the cuttings remained herbivore free as controls. Ten days later, we

removed the caterpillars, and counted the regrowth shoot number 2 weeks after the inoculation.

In 2010, we also conducted a similar field census in Finland. Because only small trees were available (average 7-cm basal diameter), we randomly selected 25 trees at each of nine sites. At one time point between late June and early July, we recorded the number of new regrowth shoots at each site, and calculated regrowth intensity. A community census was also conducted by beating and manual sampling.

We did not observe mammal browsing at any of the study sites. In Japan, willows occur along riparian zones and lakesides and wild mammals utilising willows are very rare. In Finland, the leaf beetles typically occur in a dry and sandy area near human-disturbed areas such as roadsides and airports. Mammal browsing is uncommon at such sites.

Feeding-preference test

We collected more than 60 *P. versicolora* adults from each of Japan and Finland sites in 2008 and 2010 respectively. We reared local populations separately in a plastic box containing fresh willow leaves in an environmental chamber at 23 °C, L : D 16 : 8 h, and subsequently collected newly oviposited eggs from each population. We reared these larvae with fresh mature leaves. Within 2 days after adult eclosion, we collected 20–40 adults of the next generation from each population for a choice test between new and mature leaves. The choice test was conducted in a Petri dish with leaf disks in an environmental chamber (Utsumi *et al.* 2009). We measured consumption area using the ImageJ (Rasband 1997–2012), and calculated preference as the ratio of new leaf consumption to total consumption. Choice tests for Japanese beetles were conducted in Japan, and those for Finnish beetles were conducted in Finland.

Genetic analysis

We examined genetic differentiation among local populations of *P. versicolora* in each country, using mitochondrial DNA sequence analysis. A partial region of the mitochondrial cytochrome oxidase subunit I (523bp) was sequenced from a total of 427 adults from all Japan and Finland sites (see Table S2). Adult samples were collected in each of Japan and Finland sites in 2008 and 2010, respectively, and were immediately transferred to 99% ethanol in the field. Individual DNA was extracted from hindlimbs using the Chelex method (Walsh *et al.* 1991). The COI region was PCR amplified using the primer pair PvJCO1UF (5'-TTA CCT GTA CTT GCA GGA GCA AT-3') and PvJCO1UR (5'-GTG GGA ATC ATT GAA CTAATC CAG-3'). The dye terminator cycle-sequencing reaction was performed using the BigDye Terminator Cycle Sequencing Kit version 3.1, and the reaction products were electrophoresed on an ABI 3130xl sequencer (Applied Biosystems, Foster City, CA, USA).

Community-manipulation experiments

The effects of herbivore insect community on willow regrowth and evolution in *P. versicolora* leaf feeding preference were demonstrated by conducting two community-manipulation experiments: (1) a specificity experiment and (2) a mesocosm selection experiment.

The specificity experiment was performed by collecting 18 willow cuttings from each of five trees at Yasu (YAS) site (35.05° N,

136.00° E) and three trees in Kuzu (KUZ) site (36.10° N, 136.27° E) in Japan during April 2011 (see Fig. S2). Both sites were included in the field census sites. All cuttings were planted individually in plastic pots with compost and grown under greenhouse conditions at CER. In mid-June 2011, the potted plants were randomly placed in a common garden at CER, and were individually covered with 1-mm polyethylene net mesh. We used seven herbivore species, and treatments were conducted at low and high densities for each species as follows: *P. versicolora* adults, three and six individuals; *P. versicolora* larvae, six and 12 individuals; *Chaitophorus saliniger* adults (Aphididae), 10 and 20 individuals; *Pterocomma pilosum* adults (Aphididae), 10 and 20 individuals; *Metasalis populi* adults (Tingidae), five and 10 individuals; *Ascotis seleraria* caterpillars (Geometridae), two and four individuals; *C. anastomosis* caterpillars (Notodontidae), two and four individuals; *Hyphantria cunea* caterpillars (Arctiidae), two and four individuals (eight replicates for each treatment). We also included herbivore-free plants as controls (16 replicates). The individual number for low-density treatment was based on average density observed in the field. We doubled the value for high-density treatment. The herbivore species were collected from natural willow stands in Shiga Prefecture, Japan. Insects were confined in the cages in early July 2011, and were removed 3 days later. The regrowth shoot number was counted 21 days following inoculation.

The mesocosm selection experiment was conducted by collecting 16 willow cuttings from each of four trees at YAS site, and four trees at Biwa (BIW) site (35.00° N, 135.91° E) in June 2008 (see Fig. S2). BIW site was also one of the field census sites. We planted 128 cuttings individually in plastic pots with soil under greenhouse conditions. In June 2009, we transported the potted willows to 16 greenhouses (0.9 m × 1.8 m × 2 m) placed in a common garden at CER. Each greenhouse had eight willow pots. We created seven types of experimental herbivore communities on each individual willow in the greenhouses (three species: *C. anastomosis* (Notodontidae), *Basilepta fulvipes* (Chrysomelidae), *P. pilosum* (Aphididae); all species combinations from one to three species communities and a control without herbivores (16 replicates for each treatment). The herbivores were collected in Shiga Prefecture. Insects were inoculated on individual willows as combinations of one *C. anastomosis* caterpillar (2nd instar), two *B. fulvipes* adults and 10 *P. pilosum* adults for each treatment in early July. Sixteen greenhouses were randomly assigned to the eight treatments with two replicates. Ten days later, we removed all insects from the trees, and counted regrowth shoot number 2 weeks after the inoculation. There were no aphid-attending ants in greenhouses.

Twenty days after insect removal, we inoculated *P. versicolora* adults (one female and one male) on each plant to examine their fitness consequence. For beetle-preference treatment, the leaf beetles were collected from YAS site (i.e. population with exclusive preference for new leaves) and BIW site (i.e. non-preference population) (Utsumi *et al.* 2009), and were maintained separately throughout one generation in an environmental chamber at 23 °C, L : D 16 : 8 h. We used second-generation adults obtained shortly after eclosion for the experiment. One half of the plants of each community treatment were assigned to beetle-preference treatment of the exclusive-preference population and the other half were assigned to the non-preference population. All adults were removed 2 weeks later, and we counted the number of next generation adults 1 month following inoculation. We estimated fitness

as half the number of next-generation adults, assuming 1 : 1 sex ratio. Relative fitness was calculated as follows: fitness of each replicate/mean fitness of each community treatment (Strauss *et al.* 2005).

Statistical analyses

Analyses of effects of herbivory and sites on regrowth intensity in the field and the greenhouse were performed using a generalised linear model (GLM) with a Gaussian distribution, and GLM with a Poisson distribution and log link respectively. Among-site variation in species composition, using presence/absence data of herbivore insect species was conducted using a permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations. The insect community and willow regrowth intensity data set was modelled applying GLM with a Gaussian distribution to examine effects of the number of herbivore species, overall abundance, species composition and latitude on regrowth intensity. The best model exhibited minimum Akaike Information Criterion (AIC) values among models with all variable combinations. For the models, species composition was summarised into scores of two-axis non-metric multidimensional scaling using presence/absence data. A relationship between regrowth intensity and leaf beetle preference, and among-population variation in the feeding preference were examined by GLM with a Gaussian distribution.

Exact tests of population differentiation based on haplotype frequencies with 100 000 steps in the Markov chain, and 1000 dememorisation steps were applied to evaluate genetic differentiation among leaf beetle populations. A correlation between F_{ST} and preference differences (Euclidean distance) matrices was examined, performing a pairwise Mantel test.

Effects of herbivore species composition and the number of species on regrowth intensity in the community-manipulation experiments were examined using a GLM with Poisson distribution and log link. To test pairwise differences in regrowth intensity among treatments, multiple comparisons were performed under Tukey's procedure. The relative fitness in the mesocosm selection experiment was analysed by modelling the data using a GLM with Gaussian distribution and log link (the best-fitting link function according to AIC values). The full model included the terms of preference, regrowth intensity, aphid treatment (i.e. presence/absence) and all interaction levels. Subsequently, the best-fit model was selected based on AIC values. Lower and upper confidence intervals for estimates of terms in the selected model were calculated from 2,000 bootstrapped resamples. The interaction between preference and aphid treatment was significant in the selected model; therefore, we generated individual models with overall data set, aphid-absent data set and aphid-present data set to estimate the fitness landscape in the three-dimensional space with axes of regrowth intensity, preference and relative fitness. In the model for relative fitness, the preference value was represented as 1 (exclusive preference population) or 0.5 (non-preference population).

Genetic data analyses were performed using ARLEQIN version 3.5 (Excoffier & Lischer 2010), and a pairwise Mantel test was performed using XLSTAT (version 2010.5.02; Addinsoft, Paris, France). R statistical software version 2.13.1 (R Developmental Core Team 2011) was used to conduct all other statistical analyses required in the study.

RESULTS

Herbivore community and plant responses

Our results showed among-site variation in regrowth intensity in the field, and confirmed that variation was elicited when plants were exposed to natural herbivory (GLM_(Gaussian): removal treatment: $F_{1,106} = 55.33$, $P < 0.0001$; site: $F_{7,106} = 4.67$, $P < 0.001$; treatment x site; $F_{7,106} = 4.23$, $P < 0.001$; Fig. 1a). In contrast to the herbivore-removal field experiment, plants across all study sites in Japan exhibited similar regrowth in response to inoculation with common herbivorous insects in greenhouses (GLM_(Poisson): inoculation treatment: log-likelihood ratio chi-square (LR) = 57.55, $P < 0.0001$; site: $LR = 5.03$, $P = 0.65$; treatment x site; $LR = 4.97$, $P = 0.66$; Fig. 1b).

In Japan and Finland, community composition of herbivorous insects significantly differed among sites (PERMANOVA: Japan, $F_{7,54} = 11.72$, $P < 0.0001$; Finland, $F_{8,214} = 4.07$, $P < 0.001$). Regrowth intensity (i.e. regrowth shoot number per current-year shoot) of each willow population was best explained by the number of local herbivore species among all models including any variables of the number of species, overall abundance, species composition and latitude (Table 1, Fig. 2a; see Table S1).

Feeding-preference test

We tested the feeding preference of the *P. versicolora* populations using a laboratory choice test. Significant among-population

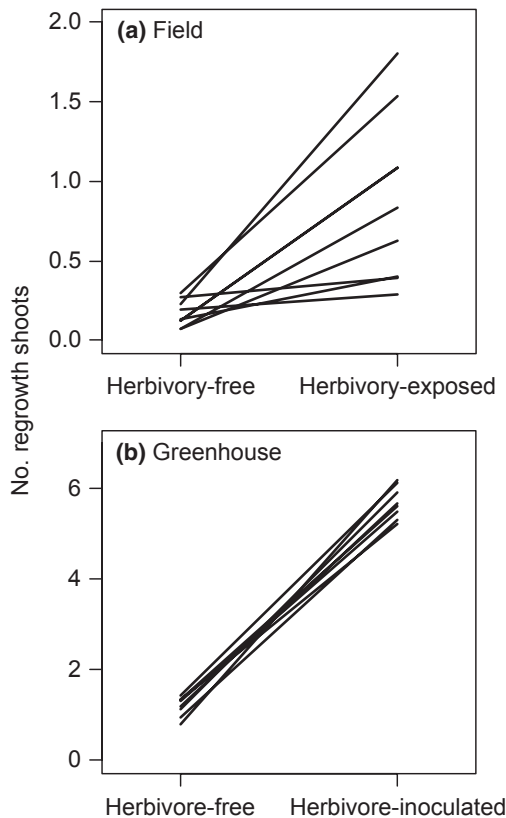


Figure 1 Willow regrowth in response to natural and inoculated herbivory. (a) Number of regrowth shoots on herbivory-exposed vs. herbivory-free shoots in the field. Each line indicates mean values at each site in Japan; (b) Effect of caterpillar inoculation on regrowth response under greenhouse conditions. Each line indicates mean values from experimental cuttings collected at each of Japan sites.

Table 1 Model selection summary for among-site variation in regrowth intensity. For Japan and Finland data, coefficients of the full model and best model are represented respectively. The best model exhibited minimum Akaike Information Criterion (AIC) values among all possible models including any of following variables: latitude, overall abundance, species composition indices and the number of species. Comp1 and 2: species composition scores by two-axis NMDS for presence/absence data. The best model indicates that regrowth intensity was predicted by the number of species in both countries (Japan: $F_{1,6} = 12.16$, $P = 0.01$; Finland, $F_{1,7} = 18.71$, $P < 0.01$). See Table S1 for the full description of model selection

	Variables					AIC
	Latitude	Abundance	Comp1	Comp2	No. species	
Japan						
Best	–	–	–	–	0.092	10.3
Full	0.277	–0.0001	–0.988	–0.379	0.105	15.9
Finland						
Best	–	–	–	–	0.045	–3.5
Full	–0.030	0.0003	–0.407	0.349	0.006	1.0

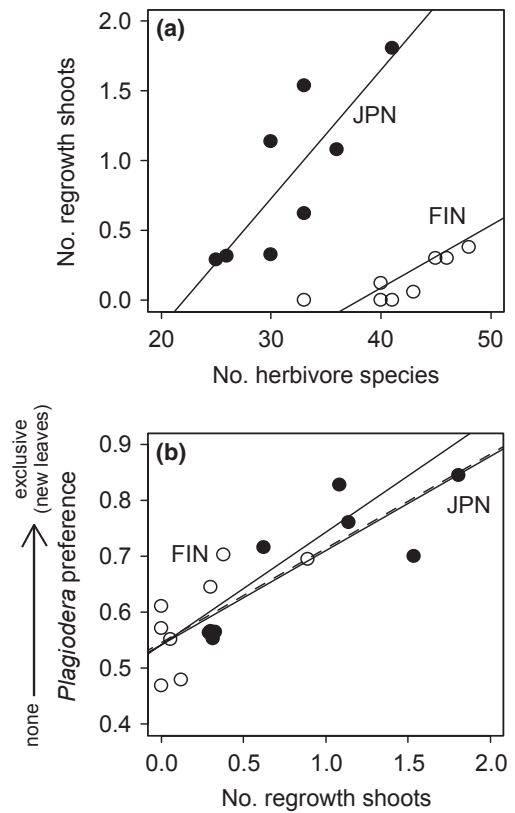


Figure 2 Relationships among the number of herbivore species, willow regrowth intensity and *P. versicolora* feeding preference. (a) Relationship between the number of species of local herbivore insect communities and willow regrowth intensity (JPN, $r^2 = 0.61$; FIN, $r^2 = 0.69$); and (b) Relationship between willow regrowth intensity and leaf beetle preference for leaf-age types (JPN, $r^2 = 0.65$; FIN, $r^2 = 0.40$). Preference value indicates a proportion of new leaf consumption to total area consumption in a choice test. The dashed line indicates predicted values from the model analysing the entire data set of both Japan and Finland sites ($r^2 = 0.68$). Each point indicates a site.

variation was detected in populations from each country (GLM_(Gaussian): Japan, $F_{7,155} = 2.95$, $P < 0.01$; Finland, $F_{8,348} = 2.24$, $P = 0.023$), and preference was associated with re-

growth intensity (Japan, $F_{1,6} = 13.79$, $P < 0.01$; Finland, $F_{1,7} = 6.45$, $P = 0.038$, Fig. 2b). Interestingly, throughout Japan and Finland, preference was consistently explained by regrowth intensity; however, no significant country effect was found (GLM_(Gaussian): regrowth, $F_{1,14} = 22.29$, $P < 0.001$; country, $F_{1,14} = 0.10$, $P = 0.75$, Fig. 2b).

Genetic analysis

A total of 38 haplotypes of *P. versicolora* were detected (see Table S2) and significant genetic differentiation was revealed among populations in each country (Exact test: Japan, $F_{ST} = 0.38$, $P < 0.0001$; Finland, $F_{ST} = 0.11$, $P < 0.0001$), indicating restricted gene flow among populations. Pairwise F_{ST} and preference differences between populations were not significantly correlated (Mantel test: Japan, $r = 0.20$, $P = 0.41$; Finland, $r = -0.27$, $P = 0.29$). We also confirmed selective neutrality of the COI region sequence evolution with Tajima's D (4.28, $P = 1.00$) and Fu's F_s (25.27, $P = 0.98$).

Community-manipulation experiments

In the specificity experiment, we found that regrowth intensity was significantly dependent on species identity of herbivores, whereas the density and interaction effects were not significant (GLM_(Poisson): species, $LR = 309.76$, $P < 0.0001$; density, $LR = 3.33$, $P = 0.07$; interaction, $LR = 11.58$, $P = 0.11$; Fig. 3). The geometrid *A. selenaria* and the notodontid *C. anastomosis* caterpillars significantly elicited regrowth response, and the willow's response to attack by the former was greater than its response to the latter species ($P < 0.05$, Tukey test; Fig. 3). However, Arctiid caterpillar *H. cunea* did not induce a regrowth response. Likewise, feeding by *P. versicolora* adults or larvae did not significantly induce willow regrowth. Hereafter, we refer to species which significantly enhanced willow regrowth response in comparison with herbivore-free treatment as 'regrowth-inducers'. Increase in density of each of regrowth inducers (*A. selenaria* and *C. anastomosis*) did not significantly affect regrowth intensity.

The mesocosm selection experiment provided the following results. First, the number of species and composition significantly affected regrowth shoot number (GLM_(Poisson): the number of

species, $LR = 150.55$, $P < 0.0001$; composition_(within the number of species), $LR = 310.04$, $P < 0.0001$), and regrowth intensity increased with the number of species (Fig. 4a). The two chewing insects, *C. anastomosis* and *B. fulvipes*, were strong and weak regrowth inducers, respectively, but the aphid, *P. pilosum*, had no effect on regrowth expression (Table 2, Fig. 4b). The beetle and caterpillar additively increased regrowth intensity, relative to the single-species treatments ($P < 0.05$, Tukey test; Table 2, Fig. 4b). Second, selective values of the leaf beetle preference were largely dependent on regrowth intensity and aphid presence/absence (Table 2, Fig. 4c, d, e). This response to selection was shown by an experimental population composed of the exclusive-preference population and the

Table 2 Analysis of (a) species composition effects on regrowth intensity and (b) fitness landscape of leaf beetle preference. Estimates refer to the estimated parameter values for a given term in (a) GLM_(Poisson) model for regrowth intensity, in which aphid, beetle and caterpillar treatments were represented by 1 (presence) or 0 (absence); and (b) the best GLM_(Gaussian) with log link model for relative fitness, in which the preference value is represented by 1 (exclusive for new leaves) or 0.5 (non-preference), and aphid treatment is represented by 1 (presence) or 0 (absence). Lower and upper confidence intervals (CIs) for each estimate were calculated from 2000 bootstrapped resamples. Bold refers to effects whose bootstrapped 95% CIs do not overlap zero

Variables	Estimates	95% CI	
		Lower	Upper
(a)			
Aphid (A)	-0.826	-16.705	17.456
Beetle (B)	1.665	0.042	17.945
Caterpillar (C)	2.995	1.731	19.368
A × B	-0.934	-17.967	16.414
A × C	-0.427	-17.246	16.953
B × C	-1.284	-17.496	0.418
A × B × C	0.836	-16.470	17.885
(b)			
Preference	-.230	-.627	-.163
Regrowth	-.052	-.086	-.023
Aphid	.452	.038	.923
Pref × Regrowth	.065	.030	.104
Pref × Aphid	-.569	-1.176	-.034

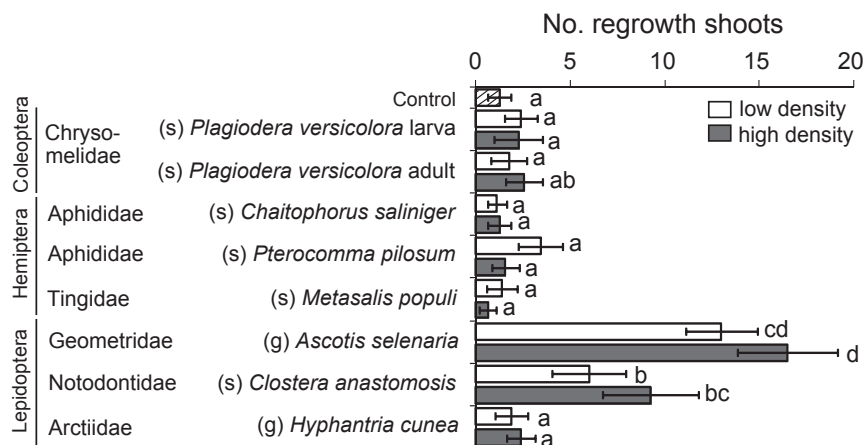


Figure 3 Specificity of willow regrowth response to attack by herbivore insect species at low and high densities. Bars are mean \pm SEM ($n = 8$, control: $n = 16$). Different letters indicate significant differences ($P < 0.05$). Letter in parentheses at the head of a herbivore's name indicates diet breadth; s: specialist (i.e. exclusively feeding on Salicaceae species, g: generalist (i.e. feeding on plant species in several families).

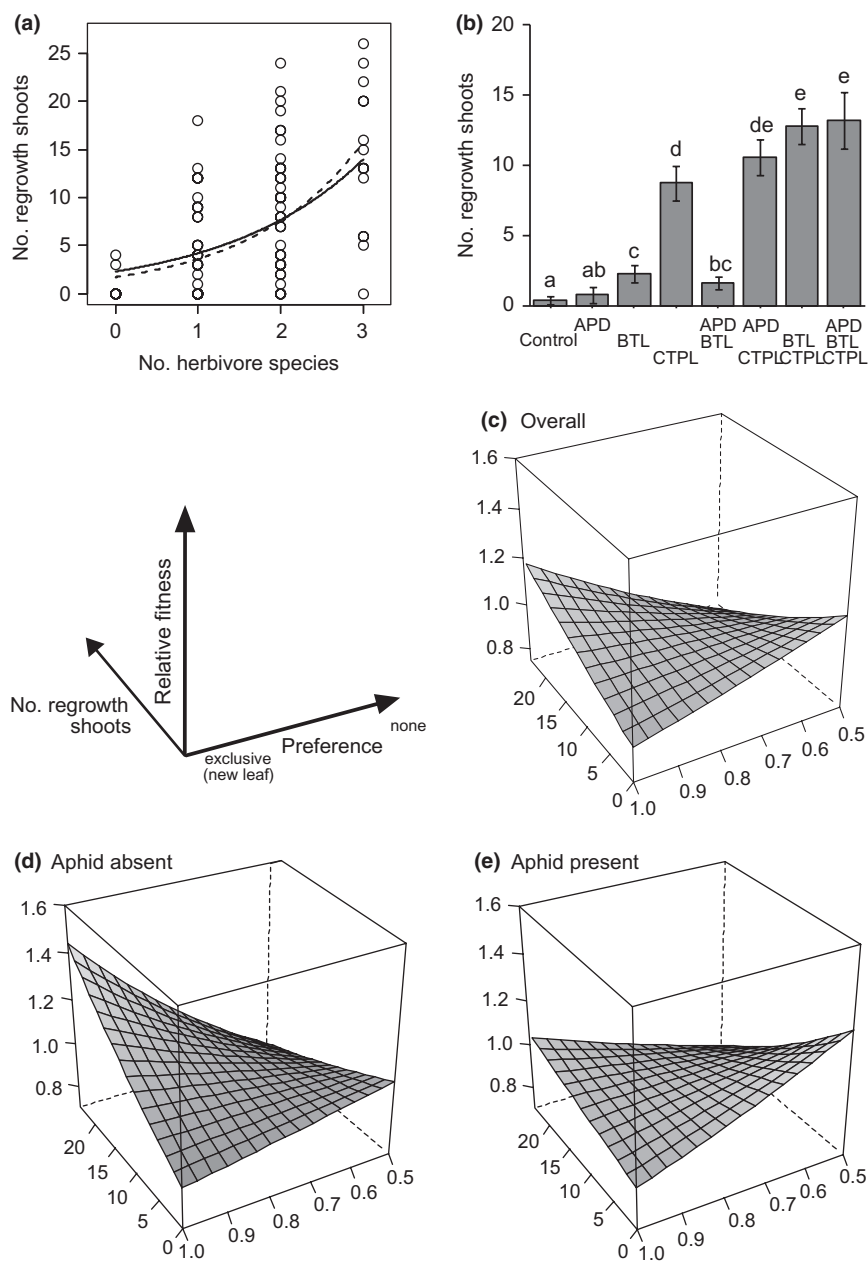


Figure 4 Results of mesocosm selection experiment. (a) Relationship between the number of herbivore species and willow regrowth. The dashed line indicates predicted values when the data set included herbivore-free treatment, and the solid line indicates predicted values from the model analysing data set without herbivore-free treatment. (b) Among-community differences in willow regrowth. APD: aphid, BTL: beetle, CTPL: caterpillar. Bars are mean \pm SEM. Different letters indicate significant differences ($P < 0.05$). (c) Fitness landscape of *P. versicolora* with respect to their preference and willow regrowth, using entire data set, (d) fitness landscape in the absence of aphids and (e) fitness landscape in the presence of aphids. Lines from left to right on the surface can be interpreted as predicted selection gradients along regrowth intensity.

non-preference population. The selection magnitude and direction was dependent on structure of the surrounding herbivore communities. Regrowth inducers indirectly imposed directional selection towards exclusive preference for new leaves through regrowth expression. On the other hand, the aphid imposed a reverse selection towards non-preference (Table 2). Overall, selection direction shifts from non-preference to exclusive preference for new leaves according to regrowth intensity (Fig. 4c). Although the fitness landscapes were generally similar between the absence and presence of

aphids, the aphid expanded the region in which non-preference was favoured (Fig. 4d, e). In addition, the sources of willows and leaf beetle populations (YAS or BIW) did not affect relative fitness (see Table S3).

DISCUSSION

Our findings across field censuses, genetic analyses and community-manipulation experiments consistently support our hypothesis that

local herbivore community composition influences the selective force on the leaf beetle *P. versicolora*, resulting in preference diversification. We demonstrated a novel indirect process linking community and trait evolution mediated by herbivore-induced plant responses.

Evolutionary patterns in the field

The field census showed among-site variation in regrowth intensity when exposed to natural herbivory. However, we detected neither variation in regrowth in the absence of herbivory in the field nor variation in regrowth in response to the same herbivory under greenhouse conditions (Fig. 1). Therefore, even assuming the presence of willow's genetic variation among populations and a heterogeneous abiotic environment, among-site variation in herbivore-induced regrowth response in the field was principally governed by local herbivory environments. Furthermore, regrowth intensity was best explained by the number of herbivore species rather than the overall abundance and/or species composition of herbivores.

As our previous study suggested (Utsumi *et al.* 2009), herbivore-induced regrowth involved phenological changes in willow leaf production, increasing the availability of new leaves through seasons (see Fig. S3), which in turn could promote exclusive preference for new leaves in *P. versicolora*. Among-population differentiation in the preference was determined by genetic effects. This is because we measured preference value by administering a laboratory choice test using offspring reared under the same conditions. In addition, we note that preference values in Japan populations in this study were consistent with our study in 2006 (Utsumi *et al.* 2009). Consequently, allopatric populations of the leaf beetle *P. versicolora* develop genetically determined divergent feeding preference according to local regrowth intensity of willows.

Although we detected genetically determined variation in the preference among the leaf beetle populations, the mitochondrial DNA sequence analyses were an integrant component of this study. This is because it is necessary to distinguish migration effects on among-population trait variation in the leaf beetle. Feeding preference often influences local colonisation when herbivores have high dispersal ability (Hanski & Singer 2001). If the leaf beetle has a preference-oriented high dispersal, leaf beetles may individually migrate into a local area where preferable resource types are abundant (Hanski & Heino 2003). This may generate among-population variation in the preference and a consequent gradient along regrowth intensity. However, our genetic analyses detected restricted gene flow among sites and found no correlation between among-population pairwise F_{ST} and variation in the preference. These results do not support migration effects on among-population trait variation. McCauley *et al.* (1988) reported similar results with allozyme markers on this beetle species in the USA, showing restricted gene flow with significant genetic variation among sites or closely spaced individual trees. Therefore, we conclude that *P. versicolora* populations have evolved divergent preference through local adaptation.

In addition, the same pattern was evident in Japan and Finland despite the different willow species in the two distant countries. This suggests that the community effects on trait evolution of a community member via induced plant responses could be widespread in plant-associated insect communities.

Evolutionary process: experimental test

Our community-manipulation experiments provided further evidence of the effects of herbivore community composition on evolution in leaf beetle preference via induced plant regrowth.

First, we discuss how herbivore community regulates willow regrowth intensity. The specificity experiment demonstrated that herbivore species identity was important for the degree of willow regrowth induction. These species-specific induction mechanisms might have resulted from a fine-tuned plant reaction against several characteristics of herbivore species, including diet breadth (e.g. specialist/generalist), feeding mode (e.g. chewer/sap-feeder), feeding position (e.g. apical/lower region of shoots) and saliva composition (Agrawal 2000; Kessler & Halitschke 2007; Rasmann & Turlings 2007). Note that feeding by *P. versicolora* adults or larvae did not significantly induce willow regrowth, indicating that regrowth expression is dependent on attack by other herbivore species. Willows would respond to *P. versicolora* attack by phenolic glycoside induction rather than regrowth response (Fields & Orians 2006).

Moreover, the mesocosm selection experiment detected significant effects of species composition and the number of species on willow regrowth intensity. The most plausible mechanism for this result is likely to be both: (1) the species-specific function for the degree of regrowth induction and (2) the integration of multiple regrowth-inducer species in a community. Importantly, the integration of any of three herbivore species (i.e. the strong regrowth inducer, the weak regrowth inducer and the non-inducer) was additive for regrowth intensity (Table 2, Fig. 4b), although density was not significant in the specificity experiment. This is probably due to different feeding habits of herbivore species within individual plants, such as feeding on different tissue types and positions (Cates 1980; Roslin *et al.* 2006), and consequently utilisation by different herbivore species, which possess different niche requirements, may additively enhance plant regrowth response. Furthermore, a more diverse community should have a higher probability of integrating multiple regrowth inducers, resulting in greater regrowth intensity of willows. While the three-species community always contained the two regrowth inducers in this experiment, the two-species-community composition varied from a community including 1 weak regrowth inducer to a community including two regrowth inducers. This resulted in a positive relationship between the number of species in a community and regrowth intensity. The above mechanism can also explain the pattern observed in the field. In the field, the occurrence of any of all species used in the two experiments was not associated with willow regrowth intensity. Willows can exhibit regrowth in response to a wide range of species, including not only folivores but also gall makers and stem borers, in the field (Nakamura *et al.* 2003; Utsumi & Ohgushi 2007). Because different herbivore species often have a similar function in terms of the degree of regrowth induction, the same regrowth intensity in local sites does not necessarily correspond to the same community composition. Therefore, in the field, the community composition scores explained regrowth intensity less well than did the number of species.

Second, we discuss how community composition shapes the selection regime for leaf beetle preference. The mesocosm selection experiment is the first to test herbivore community effects on trait evolution in a member of that community. Although ideally, several leaf beetle strains from study sites should have been applied

to such a selection experiment, unfortunately we could use a population composed of only two strain populations. However, consistently with the field pattern, the overall fitness landscape clearly showed that the direction and magnitude of selection covaried with willow regrowth intensity (Fig. 4c). Individuals with non-preference benefitted from selection imposed by a weak regrowth environment. With increase in regrowth intensity, the magnitude of the selection gradually decreased and the direction changed from non-preference towards exclusive preference for new leaves. However, we found that the non-inducer aphid, *P. pilosum*, imposed reverse selection towards non-preference, although fitness landscapes were generally similar in the presence and absence of the aphid (Table 2, Fig. 4d, e). Because the aphid colonises the growing apical region of shoots (Moritsu 1983), these individuals might have reduced quality of new leaves (Mewis *et al.* 2006). Subsequently, reduction in new-leaf quality may negatively influence the leaf beetles which have exclusive preference for new leaves, but not affect leaf beetles with non-preference. Thus, not only the integration of regrowth-inducer species but also the presence/absence of the aphid can shape the selection regime. However, in the field, the presence or absence of the aphid was unlikely to determine among-population divergence in leaf beetle preference. The occurrence of the aphid was very common across the study sites. This may be because homopteran species are thought to have a high dispersal capacity and host specificity (Strong *et al.* 1984), and the spatial turnover of herbivorous insect species among local communities is often due to generalists, but specialists widely track host plants (Briese *et al.* 1994; Lewinsohn *et al.* 2005). The aphid used in this experiment is a specialist to willows. Therefore, divergence in the leaf beetle preference would have been selected by the balance between the aphid effect that is relatively constant across sites, and the integration of multiple regrowth-inducer species that is variable among sites.

Implication for eco–evolutionary dynamics

In this system, local community structure would be expected to be temporally variable. However, local regrowth intensity may be relatively consistent enough to select for local adaptation because regrowth intensity would not be determined by the occurrence of one particular species, but by the integration of multiple regrowth-inducer species. Furthermore, a growing body of evidence has reported that trait evolution can occur in a time scale comparable to ecological changes in nature (Thompson 1998; Reznick & Ghalambor 2001; Yoshida *et al.* 2003; Grant & Grant 2006; Turcotte *et al.* 2011). We detected high heritability (0.41) and additive genetic variance in this trait in one leaf beetle population (Utsumi *et al.* 2009), although additive genetic variance should be depleted if a trait is exposed to continued directional selection (Falconer & Mackay 1996). This implies that the preference of each population may have been exposed to temporally variable selection force. Along with high heritability and genetic variation within a population, individual females of the leaf beetle repeatedly mate and lay eggs, and the leaf beetle passes several generations within a year. All of these conditions suggest that each population can rapidly evolve between non-preference and exclusive preference in response to changes in local community composition. Among-site variation may reflect temporal dynamics between community structure and trait evolution (Thompson 2005).

This study throws light on a novel process of trait evolution, involving trait-mediated indirect interactions and overall community composition. Future studies bridging the gap between ecological communities and evolution should pay attention not only to the interactions of a few members within communities but also trait-mediated indirect interactions and overall community composition.

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STATEMENT OF AUTHORSHIP

SU had the original idea for the research, and TO provided overall advice for this project. SU, YA and HR conducted the field census and laboratory work. SU and JT performed genetic analyses. SU and YA conducted common garden experiments. SU performed statistical analyses. SU wrote the draft of the manuscript, and all authors contributed substantially to revisions.

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