LETTER

Evolution of feeding preference in a leaf beetle: the importance of phenotypic plasticity of a host plant

Shunsuke Utsumi,* Yoshino Ando and Takayuki Ohgushi

Center for Ecological Research, Kyoto University, Otsu, Shiga 520-2113, Japan

***Correspondence: E-mail:**
shun@ecology.kyoto-u.ac.jp

Abstract

Much attention has been paid to ecology and evolution of damage-induced plant responses. Recently, it has been emphasized that phenotypic plasticity, such as induced plant responses, has the potential to lead to evolutionary changes of interacting partners. Here, we report that induced plant regrowth promotes a locally adaptive feeding preference of a leaf beetle, *Plagiodera versicolora*. We found that there was among-population variation in the strength of the feeding preference of the leaf beetle for leaf-age types of conspecific host plants. The strength of the preference was positively correlated to leaf production of host plants across populations, and the intensity of induced regrowth was likely to have been responsible for geographic variation in new leaf production. Within one population, we detected a significant additive genetic variance and heritability in the preference for consuming new vs. old leaves. Moreover, the strength of preference was significantly related to egg production depending on the leaf-age types. Thus, allopatric populations can evolutionarily develop different adaptive preference, according to locally distinct patterns of induced host regrowth.

Keywords

Animal–plant interaction, compensatory regrowth, geographic variation, induced plant response, local adaptation, *Plagiodera versicolora*, quantitative genetics, *Salix*.

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INTRODUCTION

Terrestrial plants show a wide range of phenotypic changes in response to biotic and abiotic damage (Karban & Baldwin 1997; Ohgushi 2005). In the past decade, researchers have revealed that the abundance, survival and reproduction of herbivorous insects are greatly influenced by induced changes in plant traits (Karban & Baldwin 1997; Ohgushi 2005). Recently, it has been emphasized that phenotypic plasticity, such as herbivore-induced plant responses, has the potential to lead to evolutionary changes in other members within communities (Agrawal 2001; Fordyce 2006). Several studies have suggested that herbivorous insects evolutionarily adapt their resource-utilizing traits against herbivore-induced plant responses (e.g. Bolter & Jongsma 1995; Broadway 1995; Chambers *et al.* 2007). However, few studies to date have examined how the induced phenotypic responses of plants affect the local adaptation of the resource-utilizing traits of herbivorous insects. As induction of plant phenotypic plasticity depends on local environmental factors (Karban & Baldwin 1997; Fordyce 2006), herbivores are likely to develop divergent traits as a local

adaptation in response to different induction of the plant plasticity.

Because herbivores have to cope with a rapidly changing suite of defensive and nutritive attributes in their host plants, they often exhibit feeding preference for a particular age of leaf (Cates 1980; Raupp & Denno 1983; Bingaman & Hart 1993; Boege & Marquis 2005). Leaf age and induced responses are major sources of variation in quality in plants. Leaf nitrogen and water content often decreases with leaf age (Raupp & Denno 1983; Denno *et al.* 1990), and the concentration of secondary compounds acting as feeding deterrents, toxins or digestibility-reducers changes depending on the leaf age (Cates 1980; Denno *et al.* 1990; Bingaman & Hart 1993). Moreover, induced plant responses often yield variation in terms of nutritional status, secondary substances and physical characteristics of host plants (Karban & Baldwin 1997; Ohgushi 2005). It should also be noted that both biotic and abiotic damage often induces compensatory regrowth of host plants (Strauss & Agrawal 1999). Induced regrowth can increase the abundance of new leaves and prolong the period of new leaf production in a host plant because of enhanced production of young plant

tissues (Nakamura *et al.* 2003). Preference and performance of herbivores are often influenced by induced regrowth (Pilson 1992; Martinsen *et al.* 1998).

The evolution of herbivore preference is influenced by relative abundance of plant species or plant tissues (Rausher 1982; Bernays 1998; Sotka *et al.* 2003; but see Thompson 1993). Theory suggests that when more favourable food types are rare, herbivores are under selection to broaden the range of food types and to utilize less favourable hosts (Rausher 1985; West & Cunningham 2002). When favourable food is abundant, strong preference for the food type can evolve because concentrating on one food type can increase the efficiency of food utilization in heterogeneous environments (Dukas & Ellner 1993; Bernays & Funk 1999). Whereas mature leaves would be ubiquitously abundant, young rapidly sprouting leaves are an ephemeral resource and inconspicuous in time and space (Cates 1980). However, induced regrowth of a host plant may alter the relative abundance and predictability of new leaves (Nakamura *et al.* 2003; Utsumi & Ohgushi 2008). For example, new lateral shoot production in response to damage by a gall midge increased new leaf production of willows from spring to summer, whereas non-galled shoots rarely produced lateral shoots or new leaves in summer (Nakamura *et al.* 2003). Hence, evolution of the feeding preference for leaf age may be affected by induced plant regrowth. Moreover, different feeding preferences among populations can develop as a local adaptation when relative abundance of a plant species or tissues differs locally (Rausher 1982; Sotka *et al.* 2003). Thus, we predict that the strength of feeding preference for new leaves increases with the local intensity of induced plant regrowth.

The willow leaf beetle, *Plagiodera versicolora* (Laicharting; Coleoptera: Chrysomelidae), is a specialist herbivore on salicaceous trees. Several authors have reported that the adult beetles exclusively prefer new leaves to mature leaves

(Raupp & Denno 1983; Ikonen 2002; Wait *et al.* 2002), although larvae feed on mature leaves. Because older leaves are of poorer quality for the beetle, egg production decreases when beetles feed on older leaves (Ishihara & Ohgushi 2006). The abundance of new leaves varies seasonally because willows sprout leaves in spring, and stop sprouting from late June to August. Thus, new leaves available to adult beetles are more likely to be limited in summer. However, three to four generations of adults emerge on willow trees continuously from spring to autumn, and thus adult females require their food resources to repeatedly produce eggs. The exclusive feeding preference for new leaves is likely to be a disadvantage because of food shortage in summer.

We investigated whether induced regrowth of willows drives local adaptation of the feeding preference of the leaf beetle *P. versicolora*. To test this hypothesis, we first documented geographic variation in the feeding preference of the beetle and induced willow regrowth over central Japan. Second, using a quantitative genetics method, we estimated the genetic basis for feeding preference in the laboratory. Finally, we obtained data showing that the strength of the feeding preference for new leaves was related to egg production.

MATERIAL AND METHODS

Geographical variation in the feeding preference

Salix eriocarpa (Franch. et Savat.) is a common willow species in central Japan ,and frequently occurs on floodplains. The eight study sites were located on riversides or a lakeside in central Japan (Fig. 1). *Salix eriocarpa* is one of the dominant species among willow stands in all of these study sites.

To examine how the feeding preference of leaf beetle adults varies among populations, we conducted a feeding
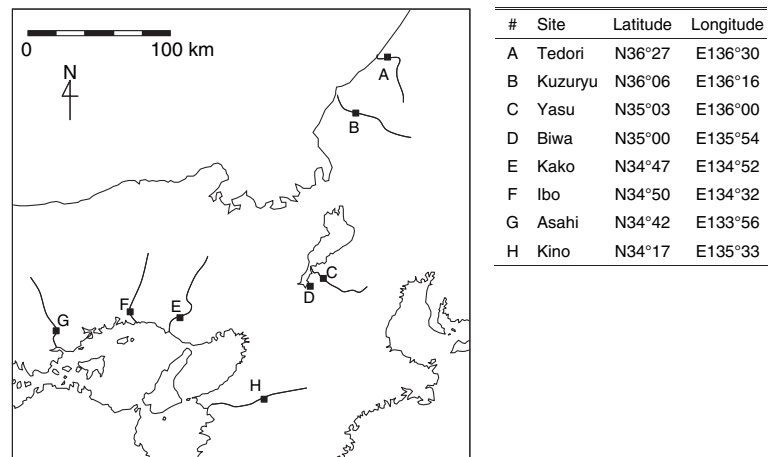

#	Site	Latitude	Longitude
A	Tedori	N36°27	E136°30
B	Kuzuryu	N36°06	E136°16
C	Yasu	N35°03	E136°00
D	Biwa	N35°00	E135°54
E	Kako	N34°47	E134°52
F	Ibo	N34°50	E134°32
G	Asahi	N34°42	E133°56
H	Kino	N34°17	E135°33

Figure 1 A map of the locations of eight sites used in this study in central Japan.

assay in the laboratory. In each of the eight study sites, we randomly selected 10 trees and collected 100 adult beetles from these trees in early July 2006. The sampled adults were put in a cool box and brought to the laboratory within 2 days. The leaf beetle adults from each site were placed together on a wet filter paper in a plastic container (20 × 15 × 5cm) and reared in an environmental chamber at 23 °C, LD 16 : 8 h for 2 weeks prior to the assay. The adults were provided new and mature leaves mixed in a proportion of 4 to 1, which is generally observed in the field. These leaves were collected from 20 trees of *S. eriocarpa* at Yasu and replaced every other day. We conducted a choice test to determine the feeding preference of the leaf beetle adults between new and mature leaves, offering leaf discs to each adult. Leaf discs were cut out with a cork borer (7 mm in inner diameter) from fresh leaves of *S. eriocarpa* from Yasu. Leaves at the third to fourth leaf nodes from the top of growing shoots were used for new leaves, and leaves at the eighth to tenth nodes were used for mature leaves. Two discs of new and mature leaves were placed in a square (4 cm × 4 cm) on wet filter paper in a Petri dish (8.5 cm in diameter). Fifty adults from each site were individually placed at the centre of the experimental Petri dish. These dishes were placed in an environmental chamber at 23 °C in a dark condition for 12 h. After 12 h, all leaf discs were removed and visually scored for the percentage of leaf consumption area at intervals of 10%. Afterward, we calculated the consumption area eaten by the beetles. To confirm that the visual estimation was accurate, we randomly selected 20 experimental leaf disks and measured the consumption area using the public domain NIH Image program (http://rsb.info.nih.gov/nih-image/). There was no significant difference in the estimate of leaf consumption between the two methods (paired *t*-test, $t = -0.20$, $P = 0.84$). To examine differences in the preferential consumption between new and mature leaves, a paired *t*-test was performed between new and mature leaves for each site. Statistical significance was Bonferroni-adjusted. Furthermore, we calculated the new leaf consumption rate as $[C_n/(C_n + C_m)]$. C_n and C_m indicate the mean consumption area of new and mature leaves, respectively. Because the assumption of normality was not met, the new leaf consumption rate was square-root transformed and then analysed using ANOVA to examine the effect of sites.

Relationship between feeding preference and new leaf production

To examine how adult-feeding preference is related to new leaf production in each site, we conducted a field survey in early July 2006 to investigate new leaf production in each site. We randomly selected eight similar-sized trees of *S. eriocarpa* spread over 400 m along a riverside or lakeshore

in each site, and randomly selected eight 1-year shoots in each tree. We defined new leaves as leaves that (i) were at the first to fifth leaf nodes from the top of a growing current-year shoot; (ii) were not fully expanded (< 80% expansion) and (iii) had bright colour tone. The other leaves were classified as mature leaves. For each 1-year shoot, we recorded the number of current-year shoots with or without new leaves. We then calculated the proportion of current-year shoots producing new leaves per tree. We used a regression analysis to examine relationships between beetle preference and proportion of shoots producing new leaves. For the analysis, the new leaf consumption rate from the laboratory experiment was square-root transformed and then regressed against the proportion of new-leaf-producing shoots.

Seasonal changes in new leaf production and induced regrowth

We compared seasonal changes in new leaf production of willows between Yasu and Biwa, as the strength of beetle preference for new leaves was apparently different between the sites (Fig. 2). To compare the seasonal occurrence of new leaf production of willows between the sites, we
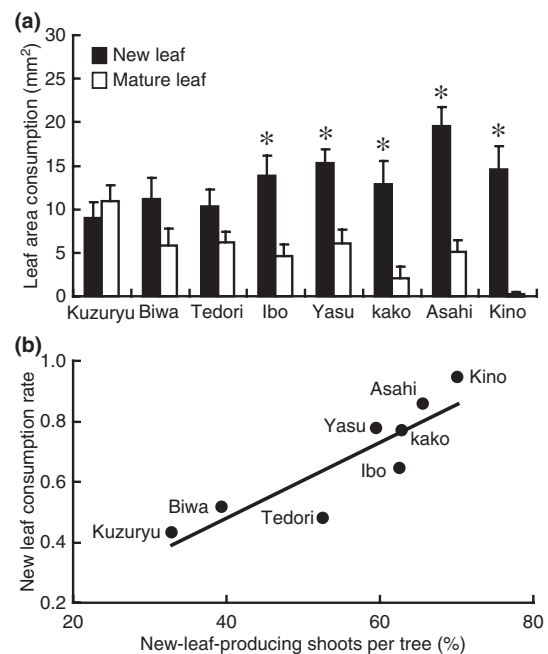

Figure 2 Among-population variation in feeding preference. (a) Leaf area consumption of new and mature leaves in the choice test for the eight local populations of the leaf beetle. For the choice test, willow leaves were collected from Yasu. An asterisk indicates a significant difference after Bonferroni correction. (b) The relationship between the rate of new leaf consumption in the choice test and the proportion of new-leaf-producing shoots per tree.

conducted a field survey once or twice every month from early June to late September 2006. Every census, we recorded new leaf production on eight trees that were randomly selected in each site. The data of the proportion of new-leaf-producing shoots were arcsine square-root transformed because the assumptions of normality were not met. We analysed the data using a repeated-measures analysis of variance (ANOVA) to test the effects of season and sites. We also compared new leaf production between Yasu and Biwa in each census using a *t*-test. We recorded the number of newly emerged lateral shoots in late June and early July. For each tree, we calculated the mean number of newly emerged lateral shoots per current-year shoot. The data were square-root transformed because of unequal variance and analysed using a *t*-test with individual trees taken as replicates.

To examine how herbivore-induced regrowth contributed to new leaf production and its variation between sites, we conducted a herbivore-removal experiment in Yasu and Biwa from early May to July 2008. In early May, we randomly selected each of the 10 intact 1-year shoots from each of the eight trees used for the survey in 2006, half of which were covered with a polyethylene mesh (1.0 mm) bag to remove herbivory. The rest of 1-year shoots were assigned as herbivore-exposed shoots on each of the trees. Prior to the treatment, we confirmed that no lateral shoots emerged. In mid-July, the number of current-year shoots, newly emerged lateral shoots and new-leaf-producing current-year shoots were recorded. Then, we calculated the proportion of new-leaf-producing shoots for herbivore-removal and -exposed shoots in each site. These data were arcsine square-root transformed because the assumptions of normality and equal variance were not met and analysed using ANOVA with site and treatment as factors. For herbivore-exposed shoots, we recorded whether new leaves were produced by either new lateral shoots (regrowth shoots) or others (non-regrowth shoots), and calculated the relative contribution of each shoot type to new leaf production. We also examined the effects of site and treatment on new lateral shoot production using ANOVA. Prior to the analysis, the number of newly emerged lateral shoots per current-year shoot was square-root transformed. In addition, we confirmed that the effect of the mesh bag on willow growth was not significant (Table S1).

To compare an ability of induced regrowth following same herbivore attack between Yasu and Biwa trees, we conducted a herbivore-inoculation experiment in a greenhouse at the Center for Ecological Research, Kyoto University (within 15 km from Yasu and Biwa). In mid-May 2008, we randomly cut off four 1-year shoots for each of the eight trees used for the herbivore removal experiment. These cuttings were pruned into 30-cm sections and individually transplanted into 0.8-L pots containing sand. The pots were randomly placed in the greenhouse with daily

water and monthly NPK fertilizer (6:10:5; HYPONeX Japan Corp. Ltd, Osaka, Japan) supply. Half of the cuttings were assigned to herbivore-inoculated plants and the rest were herbivore-free plants. In mid-September, on each of the herbivore-inoculated plants, we inoculated one 3rd instar lepidopteran caterpillar *Clostera anastomosis* Linnaeus (Notodontidae), which was collected around the common garden in the Center for Ecological Research. *Clostera anastomosis* is a common oligophagous herbivore on salicaceous plants in Eurasia. These herbivores were removed from the experimental plants 10 days later, and the number of newly emerged lateral shoots and all current-year shoots were counted 1 month after the inoculation. The mean number of newly emerged lateral shoots per current-year shoot was calculated for each parent tree and square-root transformed. ANOVA was conducted to examine the effects of trees' original site and herbivory. A parent tree was treated as a replicate.

Genetic variance and heritability of the feeding preference

A half-sib design (Falconer & MacKay 1996) was used to estimate the additive genetic variance in adult-feeding preference for new leaves of *S. eriocarpa*. Half-sib families were prepared by mating each of 20 sires sequentially to five different dams. Males mated successfully with 2.9 females on average (range 2–5), producing 57 full-sib families. To make these families, 120 egg masses were taken from Yasu in late May 2006. Each of the egg masses was reared separately in a Petri dish at 23 °C, LD 16 : 8 h until pupation and mature leaves were provided for the larvae. One virgin female was collected from each of 100 egg masses within 12 h after adult emergence and was isolated in a Petri dish. Likewise, one male was collected from each of the rest of egg masses and was isolated. As reproductive maturation is likely to be completed *c*. 24–48 h after adult emergence, one male and one female were kept for 24 h in an empty Petri dish. For mating, each male–female pair was transferred onto wet filter paper in a plastic container and provided with eight willow leaves from upper reaches of shoots. We collected eggs laid during a week and then replaced the female with another female. Leaves were collected from Yasu and replaced every other day.

Ten to twenty offspring from each full-sib family were placed together in a plastic container and were reared until adult eclosion in an environmental chamber at 23 °C, LD 16 : 8 h. One day after eclosion, offspring were placed in an empty Petri dish. To estimate the adult-feeding preference, 5–10 offspring were randomly selected from each full-sib family and then tested in a choice test according to the protocol described above. To transform the feeding data into a quantitative trait value of feeding preference for new leaves, we calculated preference indices for individual

offspring, using (ranked score of new leaf consumption) − (ranked score of mature leaf consumption). This can quantify the continuum of the preference for new leaves as a value from negative (i.e. preference for mature over new leaves) to positive (preference for new over mature leaves). We used this value for analysis of genetic variance component and heritability.

Additive genetic variance and heritability of the feeding preference were calculated from sire (δ^2_{sire}) and dam (δ^2_{dam}) variance components. Variances were estimated using a linear mixed model with restricted maximum likelihood (REML), including the sire and the dam nested within sire as random effects. We used the lmer procedure of the lme4 package in the R statistical software (R Development Core Team 2009). Significance of the random effects was tested by running models with and without each effect and calculating likelihood ratio statistics. For offspring, additive genetic variance was estimated as $V_A = 4\delta^2_{\text{sire}}$, and heritability was estimated as $h^2 = V_A/V_P$, where V_A and V_P are the causal additive genetic and phenotypic variance components respectively. Jackknife estimates (Roff & Preziosi 1994) of heritability and additive genetic variance, along with their standard errors, were calculated by deleting each half-sib family (offspring of one sire, n = number of half-sib families) from the data one by one, recalculating the parameters each time. Using the method described by Roff & Preziosi (1994), n pseudovalues were calculated. A jackknife estimate of a genetic parameter is the mean of all pseudovalues. The pseudovalues function as replicates of the parameters, allowing the calculation of standard errors and statistical tests (Roff & Preziosi 1994). In all analyses, significant deviations of heritability and additive genetic variance (one-tailed test) from zero were identified using a one-sample t-test.

Relationship between feeding preference and egg production

We conducted an experiment to examine whether variation in the feeding preference affects reproductive output. We collected 20 egg masses from Yasu in mid-May 2005 and placed them separately in plastic containers in the laboratory. Ten to twenty hatched larvae each from 20 collected egg masses were placed together on a wet filter paper in a plastic container and were reared for c. 15 days until adult eclosion in an environmental chamber at 23 °C, LD 16 : 8 h. The larvae were provided with intact mature leaves of *S. eriocarpa* in Yasu. All adults within 24 h after eclosion were used for a choice test, according to the procedure described above. We recorded which leaf type was consumed more by each adult. In each family consisting of adults from each single egg mass, we calculated the proportion of individuals that preferred new leaves.

Afterwards, 4–10 females were randomly selected from each family. Each female was paired with a randomly selected male of other families. Half of the pairs were provided with new leaves and the rest were provided with mature leaves. Each pair was separately reared on a wet filter paper in a Petri dish in an environmental chamber at 23 °C, LD 16 : 8 h. We recorded the number of eggs laid in two weeks. For each leaf type, we calculated the mean number of eggs laid per female from each family. To examine the relationship between the feeding preference and egg production, the data were analysed using a regression analysis.

RESULTS

Geographical variation in the feeding preference

We found among-population differences in the feeding preference of adult beetles using a laboratory choice test (Fig. 2a). When given a choice between new and mature leaf disks, adult beetles from five sites showed a significant, strong preference for new leaves (Ibo, Yasu, Kako, Asahi and Kino; Bonferroni-adjusted $\alpha = 0.00625$). However, there was no significant difference in the leaf consumption between new and mature leaves from other three sites (Kuzuryu, Biwa and Tedori). The new leaf consumption rate was significantly different among populations ($F_{1,284} = 6.62$, $P = 0.01$).

Relationship between feeding preference and new leaf production

According to the data obtained by the field survey, there was a significant positive relationship between the feeding preference for new leaves and new leaf production in eight local sites (Fig. 2b). This indicates that the rate of new leaf consumption in the choice test significantly increased with the proportion of new-leaf-producing shoots per tree observed in the field ($r^2 = 0.76$, $P = 0.003$).

Seasonal changes in new leaf production and induced regrowth

Seasonal patterns in new leaf production differed between two sites. In Biwa, the percentage of new-leaf-producing shoots dropped from 62% in early June to 17% in mid-July (Fig. 3). In contrast, in Yasu, the percentage of new-leaf-producing shoots was > 47% from June to August. The proportion of new-leaf-producing shoots from June to September in Yasu was significantly greater than that in Biwa ($F_{1,14} = 7.37$, $P = 0.017$). Also, there was a significant interaction effect of season × site ($F_{6,84} = 2.26$, $P = 0.045$), indicating that the seasonal pattern of new leaf production differed between sites. In particular, in late June and July, the proportion of new-leaf-producing shoots in Yasu was
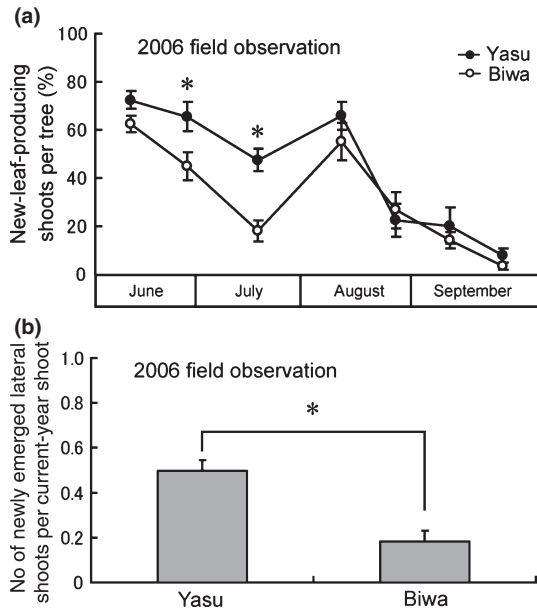
Figure 3 Difference in shoot regrowth between Yasu and Biwa. (a) Seasonal changes in the proportion of new-leaf-producing shoots per tree in Yasu and Biwa. (b) Number of newly emerged lateral shoots per current-year shoot in early summer. Vertical bars indicate SE. An asterisk indicates a significant difference ($P < 0.05$).

Figure 4 Herbivore-induced willow regrowth in Yasu and Biwa. (a) Effect of herbivore removal on new leaf production in the field. (b) Effect of herbivore removal on lateral shoot production in the field. (c) Effect of herbivore-inoculation on lateral shoot production of willow cuttings, which were taken from Yasu and Biwa, in the greenhouse. Vertical bars indicate SE. Different letters indicate significant difference (Tukey test: $P < 0.05$).

significantly greater than that in Biwa (t-test: late June, $t = 2.39$, $P = 0.03$; July, $t = 4.96$, $P < 0.001$) and there was a marginally significant difference in early June ($t = 1.96$, $P = 0.069$), although no significant differences were found in August and September ($P > 0.05$). The number of newly emerged lateral shoots with new leaves per shoot in early summer in Yasu was 2.7 times greater than that in Biwa ($t = 3.88$, $P = 0.001$; Fig. 3).

Herbivore removal significantly reduced new leaf production in Yasu, but not in Biwa (site: $F_{1,28} = 0.56$, $P = 0.46$; removal: $F_{1,28} = 11.19$, $P = 0.002$; site × removal: $F_{1,28} = 6.41$, $P = 0.017$; Fig. 4a), indicating that new leaf production was greatly enhanced when willows were exposed to herbivory in Yasu. Although the proportion of new-leaf-producing shoots was up to 50% in herbivore-exposed willows in Yasu, the proportion was c. 20% in Biwa under both herbivore-exposed and removal treatments. New leaf production largely depended on regrowth shoots over non-regrowth shoots in July. Consistent with this result, the number of newly emerged lateral shoots was significantly greater when willows were exposed to herbivory in Yasu than in Biwa (site: $F_{1,28} = 3.13$, $P = 0.088$; removal: $F_{1,28} = 15.96$, $P < 0.001$; site × removal: $F_{1,28} = 8.71$, $P = 0.006$; Fig. 4b). In Biwa, there was no significant effect of herbivore removal on willow regrowth. However, in the greenhouse experiment, cuttings
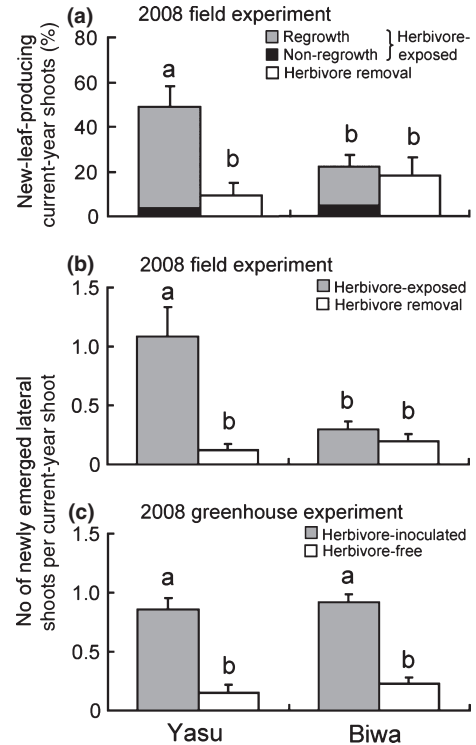
from trees in Yasu and Biwa significantly responded to herbivore inoculation, increasing the lateral shoot production to a similar level ($F_{1,28} = 1.41$, $P = 0.245$; $F_{1,28} = 57.46$, $P < 0.001$; $F_{1,28} = 0.42$, $P = 0.52$; Fig. 4c).

Genetic variance and heritability of the feeding preference

Our half-sib analysis showed that the among-sires and the among-dams (within sires) components of variation were significant (Table 1a). Our estimates of the additive genetic variance (V_A) and the heritability ($h^2 = V_A/V_P$) were 1.65 ± 0.07 (SE) and 0.41 ± 0.02 (SE) respectively, both of which were significantly different from zero (Table 1b).

Relationship between feeding preference and egg production

A feeding preference for new leaves was indicated by a larger percentage of individuals consuming new leaves than mature leaves in each family. There was a significant relationship between feeding preference for new leaves and

Table 1 Results of half-sib analysis. (a) Variance components from a liner mixed model with REML estimation for the preference index of half-sib families. We present log-likelihood ratio statistics (LR χ^2) of models with and without the effect. (b) Estimates of the additive genetic variance and heritability. Both estimates were significantly greater than zero

(a) Source	Variance components	LR χ^2	P
Sire	0.41	4.82	0.028
Dam (within sire)	0.40	4.26	0.039
Error	3.19		

(b) Components	Estimates	SE	P
V_A	1.65	0.07	< 0.001
h^2	0.41	0.02	< 0.001

egg production (Fig. 5). When adult beetles were reared on new leaves, the egg production was positively related to the strength of feeding preference for new leaves ($r^2 = 0.24$, $b = 0.60$, $P = 0.033$). In contrast, a significantly negative relationship was found when adult beetles were reared on mature leaves ($r^2 = 0.24$, $b = -0.19$, $P = 0.033$).

DISCUSSION

Recent reviews have emphasized that induced responses of plants have profound evolutionary consequences for various traits of plant-associated herbivorous insects (Agrawal 2001; Fordyce 2006). Previous studies have shown that induced plant responses have a potential to affect the evolution of resource-utilizing traits of herbivores (Bolter & Jongsma 1995; Broadway 1995; Chambers
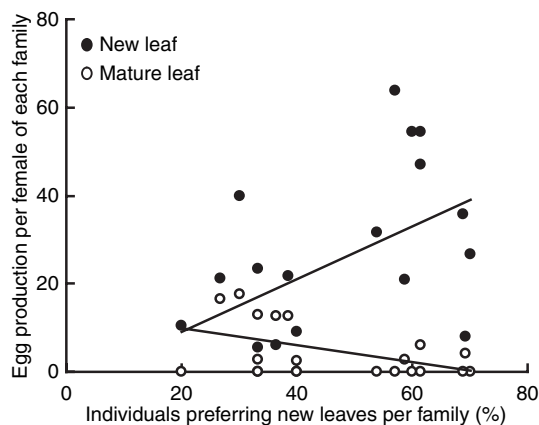

Figure 5 Relationships between a feeding preference for new leaves and egg production of the leaf beetle when fed on new leaves (•) ($r^2 = 0.24$, $b = 0.60$, $P = 0.033$) and mature leaves (○) ($r^2 = 0.24$, $b = -0.19$, $P = 0.033$). Leaf beetles and willow leaves were collected from Yasu.

et al. 2007). To our knowledge, the present study is the first report of divergent local adaptation of a feeding preference in response to the different intensity of induced plant responses.

Among-population variation in the feeding preference of the leaf beetle was closely related to new leaf production in each study site. Greater production of new leaves seems to result from enhanced production of newly emerged lateral shoots as damage-induced regrowth. This was supported by our herbivore removal experiments using willows of Yasu and Biwa. Moreover, the herbivore-inoculation revealed that willows of Biwa had the ability to produce lateral shoots in response to herbivory as well as trees of Yasu, but lateral shoot production in Biwa was not significantly enhanced when the willows were exposed to herbivory in the field. This indicates that differences in local biotic (e.g. herbivore community structure) and/or abiotic factors (e.g. soil nutritious status) are responsible for the variation in the intensity of induced regrowth (Wise & Abrahamson 2005). In particular, community composition may be more responsible for the variation, because C. anastomosis is rare in Biwa but common in Yasu. In this study, the geographic variation in new leaf production was likely to be a result of differences in the intensity of induced regrowth in response to local environmental factors. Also, as a result of the greater production of lateral shoots in Yasu than that in Biwa, new leaf production in Yasu was maintained at a higher level until August. Therefore, induced regrowth can increase temporal and spatial variability of relative abundance of new leaves. In addition, note that differences in new leaf and lateral shoot production in Yasu and Biwa were consistent between 2006 and 2008 (Figs 3 and 4). This suggests that local regrowth intensity is likely maintained over years and provides a basis for selection for divergent local adaptation of herbivores. Because large-scale disturbance by flooding (Nakamura et al. 2005) and fire (Stein et al. 1992) would also induce willow regrowth, further studies will need to explore the long-term regrowth pattern among sites.

There are three possible underlying mechanisms for the observed correlation between the beetle preference for new leaves and the intensity of host plant regrowth: (i) conditioning effect; (ii) local adaptation and (iii) colonization effect. First, the experience of herbivorous insects on a given host plant can influence subsequent feeding preference (Peacock et al. 2003; but see Via 1991). However, it is unlikely that the experience of new leaves would be responsible for the geographic variation in the present study. Offspring of Yasu and Biwa leaf beetle populations showed similar preference to those of parental populations respectively, even when beetles were reared on the same mixture of new and mature leaves through a larval and adult period (S. Utsumi, unpublished

data). Second, specialist herbivores often show fine-scale adaptation to local plant phenotypes (e.g. Karban 1989; Mopper *et al.* 2000; but see Strauss 1997). Moreover, there are dozens of examples showing that geographic variation in preference for locally abundant plant species develops as a local adaptation (Fox & Morrow 1981; Rausher 1982; Thompson 1988; Bernays & Chapman 1994; Berenbaum & Zangerl 1998; Sotka *et al.* 2003; but see Thompson 1993). Theoretical studies have suggested that when more favourable food types (higher quality) are rare or absent, herbivores are under selection to broaden the range of food types, and they utilize less favourable hosts (lower quality) (Rausher 1985; West & Cunningham 2002). When favourable food is abundant, strong preference for the food type can evolve because concentrating on one food type can increase the efficiency of food utilization in heterogeneous environments (Dukas & Ellner 1993; Bernays & Funk 1999; West & Cunningham 2002). Third, host preference influences colonization in a local area when herbivores have a high dispersal ability (Hanski & Singer 2001). By preference-biased dispersal, herbivores immigrate into a local area where preferable hosts are relatively abundant (Hanski & Heino 2003). Given their preference-biased dispersal, beetles with stronger preference for new leaves can colonize sites where willows produce new leaves more readily, although beetles with a weak preference might exhibit non-biased colonization. However, McCauley *et al.* (1988) detected significant genetic variation among groups of *P. versicolora* on individual trees. Thus, the second explanation (local adaptation) remains more plausible.

As we could not provide willow leaves from multiple sites in the choice test, we did not examine an interaction between plant origin and herbivore population. If willow traits that influence beetle preference differ among site, the different preference observed in this study might not reflect the nature of preference of each population. However, it is unlikely to occur because the quality of new and mature leaves (i.e. concentration of nitrogen, total phenolics and condensed tannin) was not different among sites (S. Utsumi, unpublished data). We also observed that the relative abundance of the adult beetles on new and mature leaves agreed to the expectation from the preference detected in the respective sites.

The additive genetic variance for the feeding preference indicates that this trait is likely to evolve in response to natural selection (Futuyma & Peterson 1985; Jaenike & Holt 1991). We found significant relationships between preference and reproductive output: a positive relationship between preference for new leaves and egg production for beetles reared on new leaves and a negative relationship when beetles were reared on mature leaves. Our results suggest that relative abundance of new leaves can work as

a selective force. *Plagiodera versicolora* adults show a strong relationship between leaf-age-related consumption rate and fecundity (Raupp 1985). Also, reproductive inactivity in these female adults is induced by a decline in host quality (Ishihara & Ohgushi 2006). Thus, feeding preference may be correlated with both consumption rate and reproductive inactivity, which in turn affect offspring production. When new leaves are available, adults with a strong preference for them would find new leaves more efficiently than adults with a weak preference. In these adults, under an environment with only mature leaves, reproductive inactivity may be temporarily induced. This is likely to be adaptive in an environment with abundant new leaves and a high predictability of new leaf recruitment. For example, Ohgushi (1995) documented adaptive oviposition of females of the herbivorous lady beetle, *Epilachna niponica*. These females resorbed eggs in the ovary when host plants, *Cirsium kagamontanum*, were damaged by flooding, and resumed oviposition immediately in response to sprouting of new leaves on damaged plants. In contrast, in adults with a weak preference, more consumption of mature leaves and less induction of reproductive inactivity may result in a greater egg production when only mature leaves are available. This is likely to be adaptive under a scarcity of new leaves and low predictability of new leaf recruitment. Hence, variation in the intensity of induced plant regrowth is critical to drive the locally adaptive feeding preference of *P. versicolora* through a change in the relative availability of new leaves.

On the other hand, the larvae prefer mature leaves to new leaves in the field. This may occur because there is high predation pressure on the larvae, severe microclimate and/or strong interspecific competition on new leaves (Raupp & Denno 1983). Additionally, because the adult females oviposit multiple times in their reproductive lifetime, for the less-mobile larvae it may be adaptive to feed on mature leaves, which are relatively abundant and stable resource until the end of season.

Our results show that induced plant regrowth can promote locally adaptive feeding preferences of herbivorous insects. This study suggests that the variation in induced willow regrowth is likely to maintain the geographic and genetic variation in the feeding preference. We would like to emphasize that induced phenotypic plasticity of a plant can play an important role in the trait evolution of herbivorous insects closely associated with it, because induced plant responses are ubiquitous in a wide range of plants (Karban & Baldwin 1997). Understanding the evolution of resource-utilizing traits of herbivorous insects requires a greater appreciation that induced plant responses determine the plant phenotypes and ultimately can alter the direction and strength of natural selection for associated herbivores.

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REFERENCES

Agrawal, A.A. (2001). Phenotypic plasticity in the interactions and evolution of species. *Science*, 294, 321–326.

Berenbaum, M.R. & Zangerl, A.R. (1998). Chemical phenotype matching between a plant and its insect herbivore. *Proc. Natl Acad. Sci. U.S.A.*, 95, 13743–13748.

Bernays, E.A. (1998). Evolution of feeding behavior in insect herbivores. *Bioscience*, 48, 35–44.

Bernays, E.A. & Chapman, R.F. (1994). *Host-plant Selection by Phytophagous Insects*. Chapman and Hall, New York.

Bernays, E.A. & Funk, D.J. (1999). Specialists make faster decisions than generalists: experiments with aphids. *Proc. R. Soc. Lond., B*, 266, 151–156.

Bingaman, B.R. & Hart, E.R. (1993). Clonal and leaf age variation in *Populus* phenolic glycosides: implications for host selection by *Chrysomela scripta* (Coleoptera: Chrysomelidae). *Environ. Entomol.*, 22, 397–403.

Boege, K. & Marquis, R.J. (2005). Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends Ecol. Evol.*, 20, 441–448.

Bolter, C.J. & Jongsma, M.A. (1995). Colorad potato beetles (*Leptinotarsa decemlineata*) adapt to proteinase inhibitors induced in potato leaves by methyl jasmonate. *J. Insect. Physiol.*, 41, 1071–1078.

Broadway, R.M. (1995). Are insects resistant to plant proteinase inhibitors? *J. Insect. Physiol.*, 41, 107–116.

Cates, R.G. (1980). Feeding patterns of monophagous, oligophagous and polyphagous insect herbivores: the effect of resource abundance and plant chemistry. *Oecologia*, 46, 22–31.

Chambers, J.L.E., Berenbaum, M.R. & Zangerl, A.R. (2007). Benefits of trenching behavior in the context of an inducible defense. *Chemoecology*, 17, 125–130.

Denno, R.F., Larsson, S. & Olmstead, K.L. (1990). Role of enemy free space and plant quality in host-plant selection by willow beetles. *Ecology*, 71, 124–137.

Dukas, R. & Ellner, S. (1993). Information processing and prey detection. *Ecology*, 74, 1337–1346.

Falconer, D.S. & MacKay, T.F.C. (1996). *Introduction to Quantitative Genetics*. Longman, New York.

Fordyce, J.A. (2006). The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. *J. Exp. Biol.*, 209, 2377–2383.

Fox, L.R. & Morrow, P.A. (1981). Specialization: species property or local phenomenon? *Science*, 211, 887–893.

Futuyma, D.J. & Peterson, S.C. (1985). Genetic variation in the use of resources by insects. *Annu. Rev. Entomol.*, 30, 217–238.

Hanski, I. & Heino, M. (2003). Metapopulation-level adaptation of insect host plant preference and extinction-colonization dynamics in heterogeneous landscapes. *Theor. Popul. Biol.*, 64, 281–290.

Hanski, I. & Singer, M.C. (2001). Extinction-colonization dynamics and host-plant choice in butterfly metapopulations. *Am. Nat.*, 158, 341–353.

Ikonen, A. (2002). Preferences of six leaf beetle species among qualitatively different leaf age classes of three Salicaceous host species. *Chemoecology*, 12, 23–28.

Ishihara, M. & Ohgushi, T. (2006). Reproductive inactivity and prolonged developmental time induced by seasonal decline in host plant quality in the willow leaf beetle *Plagiodera versicolora* (Coleoptera: Chrysomelidae). *Environ. Entomol.*, 35, 524–530.

Jaenike, J. & Holt, R.D. (1991). Genetic variation for habitat preference: evidence and explanations. *Am. Nat.*, 137, S67–S90.

Karban, R. (1989). Fine-scale adaptation of herbivorous thrips to individual host plants. *Nature*, 340, 60–61.

Karban, R. & Baldwin, I.T. (1997). *Induced Responses to Herbivory*. University of Chicago Press, Chicago.

Martinsen, G.D., Driebe, E.M. & Whitham, T.G. (1998). Indirect interactions mediated by changing plant chemistry: beaver browsing benefits beetles. *Ecology*, 79, 192–200.

McCauley, D.E., Wade, M.J., Breden, F.J. & Wohltman, M. (1988). Spatial and temporal variation in group relatedness: evidence from the imported willow leaf beetle. *Evolution*, 42, 184–192.

Mopper, S., Stiling, P., Landau, K., Simberloff, D. & Van Zandt, P. (2000). Spatiotemporal variation in leafminer population structure and adaptation to individual oak trees. *Ecology*, 81, 1577–1587.

Nakamura, M., Miyamoto, Y. & Ohgushi, T. (2003). Gall initiation enhances the availability of food resources for herbivorous insects. *Funct. Ecol.*, 17, 851–857.

Nakamura, M., Utsumi, S., Miki, T. & Ohgushi, T. (2005). Flood initiates bottom-up cascades in a tri-trophic system: host plant regrowth increases densities of a leaf beetle and its predators. *J. Anim. Ecol.*, 74, 683–691.

Ohgushi, T. (1995). Adaptive behavior produces stability in herbivorous lady beetle populations. In: *Population Dynamics: New Approaches and Synthesis* (eds Cappuccino, N. & Price, P.W.). Academic Press, San Diego, pp. 303–319.

Ohgushi, T. (2005). Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annu. Rev. Ecol. Evol. Syst.*, 36, 81–105.

Peacock, L., Carter, P., Powers, S. & Karp, A. (2003). Geographic variation in phenotypic traits in *Phratora* spp. and the effects of conditioning on feeding preference. *Entomol. Exp. Appl.*, 109, 31–37.

Pilson, D. (1992). Aphid distribution and the evolution of goldenrod resistance. *Evolution*, 46, 1358–1372.

R Development Core Team (2009). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available at: http://www.R-project.org Accessed 30 May 2009.

Raupp, M.J. (1985). Effects of leaf toughness on mandibular wear of the leaf beetle, *Plagiodera versicolora*. *Ecol. Entomol.*, 10, 73–79.

Raupp, M.J. & Denno, R.F. (1983). Leaf age as predictor of herbivore distribution and abundance. In: *Variable Plants and Herbivores in Natural and Managed Systems* (eds Denno, R.F. & McGlure, M.S.). Academic Press, New York, pp. 91–124.

Rausher, M.D. (1982). Population differentiation in *Euphydryas editha* butterflies: larval adaptation to different hosts. *Evolution*, 36, 581–590.

Rausher, M.D. (1985). Variability for host preference in insect populations: mechanistic and evolutionary models. *J. Insect. Physiol.*, 31, 873–889.

Roff, D.A. & Preziosi, R. (1994). The estimation of the genetic correlation: the use of the jackknife. *Heredity*, 73, 544–548.

Sotka, E.E., Wares, J.P. & Hay, M.E. (2003). Geographic and genetic variation in feeding preference for chemically defended seaweeds. *Evolution*, 57, 2262–2276.

Stein, S.J., Price, P.W., Abrahamson, W.G. & Sacchi, C.F. (1992). The effect of fire on stimulating willow regrowth and subsequent attack by grasshoppers and elk. *Oikos*, 66, 190–196.

Strauss, S.Y. (1997). Lack of evidence for local adaptation to individual plant clones or site by a mobile specialist herbivore. *Oecologia*, 110, 77–85.

Strauss, S.Y. & Agrawal, A.A. (1999). The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.*, 14, 179–185.

Thompson, J.N. (1988). Evolutionary genetics of oviposition preference in swallowtail butterflies. *Evolution*, 42, 1223–1234.

Thompson, J.N. (1993). Preference hierarchies and the origin of geographic specialization in host use in swallowtail butterflies. *Evolution*, 47, 1585–1594.

Utsumi, S. & Ohgushi, T. (2008). Host plant variation in plant-mediated indirect effects: moth boring-induced susceptibility of willows to a specialist leaf beetle. *Ecol. Entomol.*, 33, 250–260.

Via, S. (1991). Specialized host plant performance of pea aphid clones is not altered by experience. *Ecology*, 72, 1420–1427.

Wait, D.A., Coleman, J.S. & Jones, C.G. (2002). *Chrysomela scripta*, *Plagiodera versicolora* (Coleoptera: Chrysomelidae), and *Trichoplusia ni* (Lepidoptera: Noctuidae) track specific leaf developmental stages. *Environ. Entomol.*, 31, 836–843.

West, S.A. & Cunningham, J.P. (2002). A general model for host plant selection in phytophagous insects. *J. Theor. Biol.*, 214, 499–513.

Wise, M.J. & Abrahamson, W.G. (2005). Beyond the compensatory continuum: environmental resource levels and plant tolerance of herbivory. *Oikos*, 109, 417–428.

SUPPORTING INFORMATION

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Table S1 Examination of effect of covering in the field experiment

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