

Enemy-free space? Host preference and larval performance of a willow leaf beetle

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Abstract To examine whether enemy-free space is an important factor determining the host utilization pattern of a leaf beetle *Plagioder a versicolora*, we investigated the relationship between adult preference and offspring performance on three co-occurring willow species, *Salix sachalinensis*, *S. miyabeana* and *S. integra*. *Salix sachalinensis* was by far the most preferred host plant of feeding adults, while both *S. miyabeana* and *S. integra* were rarely fed upon. The fact that most oviposition was observed on *S. sachalinensis* also suggested that *P. versicolora* preferred *S. sachalinensis* to other willows for oviposition. This adult preference did not correspond well to patterns of larval performance on the three willow species in the absence of enemies. Higher survivorship, shorter developmental time and larger adult size were achieved on *S. sachalinensis* and *S. miyabeana* than on *S. integra*. Performance as indicated by female adult size and development time on *S. miyabeana* were higher than on *S. sachalinensis*. In the presence of enemies, however, the survivorship of first-instar larvae on *S. miyabeana* was much lower than on other willows. Adults of *P. versicolora* apparently avoided *S. miyabeana*

as an oviposition and feeding host and preferred *S. sachalinensis* as an enemy-free space. This was not because larvae had poorer performance on *S. miyabeana*, but because predation pressure on eggs and early instar larvae was more severe on *S. miyabeana*.

Keywords Bottom-up and top-down effects · Natural enemies · *Plagioder a versicolora* · Preference–performance linkage · *Salix*

Introduction

The relationship between adult host preference and offspring performance has been a central issue in the study of the evolution of insect–plant interactions (Futuyma and Peterson 1985; Thompson 1988a, b; Thompson and Pellmyr 1991). Obviously, natural selection will favor oviposition preference for host plants on which offspring perform best. The preference–performance linkage has recently been explored in terms of host plant selection or oviposition site selection on an individual plant, revealing a positive correlation (genetic or phenotypic) between oviposition preference and offspring performance (Wiklund 1975; Whitham 1980; Rausher 1982; Williams 1983; Leather 1985; Via 1986; Damman and Feeny 1988; Singer et al. 1988; Craig et al. 1989; Minkenberg and Ottenheim 1990; Ohgushi 1992, 1998; Kouki 1993; Nylin and Janz 1993; Yamaga and Ohgushi 1999; Craig and Ohgushi 2002).

On the other hand, some herbivorous insects show a poor correspondence between oviposition preference and offspring performance (Chew 1977; Smiley 1978; Rausher 1979; Courtney 1981, 1982; Messina 1982; Roininen and Tahvanainen 1989; Kagata and Ohgushi 2001). The weak preference–performance correlation may result from

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oviposition on to introduced host plants or a relative shortage of suitable plants or plant parts (Thompson 1988b), or constraints of life history characteristics such as a short adult lifespan constraining oviposition or poor capacity for directed flight (Underwood 1994; Larsson and Ekbom 1995). In addition, the effects of natural enemies on offspring survival may also result in this weak correspondence (Price et al. 1980; Courtney 1988; Thompson 1988b; Denno et al. 1990).

A number of studies on the relationship between adult host preference and offspring performance have been conducted in the absence of natural enemies under laboratory conditions (see Thompson and Pellmyr 1991). These studies have largely focused on bottom-up effects of host plant quality as primary determinants of offspring performance (Awmack and Leather 2002). However, a ranking of performance based upon survivorship and growth in the absence of natural enemies can differ from the ranking in the presence of enemies in the wild (Thompson 1988b). For example, in the willow leaf beetle *Pharatora vitellinae*, ovipositing females avoided *Salix viminalis* as an oviposition host despite high larval performance because the larvae that use plant-derived salicylaldehyde as a defensive secretion, were defenseless against generalist predators when raised on this salicylate-poor willow (Denno et al. 1990). In the white butterfly, *Pieris napi*, females preferred to lay eggs on wild crucifers belonging to the genus *Arabis*, which were the least suitable hosts for larval performance (Ohsaki and Sato 1994). This preference was adaptive because the parasitism rate was much lower on *Arabis* plants than on other crucifers.

In general, top-down effects by natural enemies on herbivores may vary among host plants (Thompson 1988b). Natural selection for utilization of host plants may therefore be partly determined by enemy-free space (Price et al. 1980, 1986). In the context of multitrophic interactions (Price et al. 1980), we need to determine the relative importance of direct and indirect effects by natural enemies on offspring performance to better understand the preference–performance linkage (Bernays and Graham 1988; Gross and Price 1988; Valladares and Lawton 1991; Yamaga and Ohgushi 1999). However, such evidence in *P. vitellinae* and white butterflies are the only cases in which the significance of enemy-free space on the determination of host utilization pattern of herbivorous insects is well understood. Thus, we need more evidence in order to be able to generalize about the importance of the top-down effects by natural enemies on the host utilization pattern of herbivorous insects.

In this study, we investigated the relationship between adult host preference and larval performance of the willow leaf beetle *Plagioderma versicolora* Laicharting (Coleoptera: Chrysomelidae) on three Japanese willow species (*Salix*) under both field and laboratory conditions. Unlike the cases

of *P. vitellinae* (Denno et al. 1990) and several species of *Chrysomela* (Pasteels et al. 1990; Rowell-Rahier and Pasteels 1990), larvae of *P. versicolora* use independently synthesized compounds, rather than plant-derived chemicals, as a defensive secretion against predators (Meinwald et al. 1977; Sugawara et al. 1979; Matsuda and Sugawara 1980; Pasteels et al. 1990). Therefore, larval defense in *P. versicolora* is not directly related to plant secondary compounds. The main objective of this study is to examine whether enemy-free space is an important factor in determining the host utilization pattern of *P. versicolora* by evaluating the relative importance of bottom-up effects due to host plant quality and top-down effects due to natural enemies on larval performance.

Materials and methods

The study species and site

The willow leaf beetle *P. versicolora* is a common herbivorous insect on willows over wide areas of Asia, Europe and North Africa (Kimoto and Takizawa 1994). In addition, *P. versicolora* was introduced into North America in 1911 (Hood 1940; Wade and Breden 1986; Wade 1994). Both adults and larvae feed exclusively on willow leaves. When larvae of *P. versicolora* are attacked by natural enemies, they secrete defensive substances from eversible glands on the dorsolateral surface of the pronotum (Meinwald et al. 1977; Sugawara et al. 1979; Wade and Breden 1986; Pasteels et al. 1990). In the Japanese strain of *P. versicolora*, the defensive secretion consists of cyclopentanoid monoterpenes, such as plagiolacton, epiplagiolactone, plagiodial and epichrysomelidial, none of which are derived from the host plant but are independently synthesized by the larvae (Sugawara et al. 1979).

The field experiments and collections were conducted along the Ishikari River (43°N, 141°E; Ishikari, Hokkaido, Japan). At this study site, six willow species (*Salix miyabeana* Seemen, *S. sachalinensis* Fr. Schm, *S. integra* Thunb, *S. subfragilis* Anders, *S. hultenii* Floderus and *S. pet-susu* Kimura) are present (Ishihara et al. 1999). In this study, we used *S. miyabeana*, *S. sachalinensis* and *S. integra* as host plants because more than 90% of all willows at the study site are one of these three species, and eggs of *P. versicolora* were found only on those. The abundance of *P. versicolora* adults and egg clutches varies among willow species (Ishihara et al. 1999). Our field census through the season indicated that most *P. versicolora* adults and egg clutches are found on *S. sachalinensis*, which is the second most abundant willow species in the study area (Ishihara et al. 1999). This evidence suggests a strong adult host preference for *S. sachalinensis* over other willow species.

However, *P. versicolora* larvae can feed on all the willow species except for *S. subfragilis* (unpubl. data).

At Ishikari, *P. versicolora* has a bivoltine life cycle (Ishihara et al. 1999). Adults that have overwintered lay egg clutches on willow leaves starting in early June. The number of eggs per clutch varies largely from 5 to 25 among clutches (mean \pm SD: 13.7 ± 4.9 , $n = 75$; unpubl. data). The hatched larvae feed in groups of related individuals, but in most cases mature (third instar) larvae feed solitarily. Pupation occurs on leaves after the third instar. The first-generation adults start emerging in mid-July and produce the second generation. The second-generation adults emerge in August when willow leaves begin to turn color. Leaves abscise from willow trees by early September. In addition to the second-generation adults, a portion of the first-generation adults that emerge late may also overwinter without reproducing (Ishihara 2000). *P. versicolora* adults enter diapause to overwinter (Hood 1940; Ishihara 2000; Ishihara and Hayashi 2000).

The laboratory experiments were conducted in incubators at the Institute of Low Temperature Science, Hokkaido University (Sapporo, Hokkaido, Japan), located 15 km to the southwest of the Ishikari field site.

Adult preference

Feeding preferences of *P. versicolora* adults were determined via a choice test in the laboratory. Eight pairs of adults were collected at Ishikari on 17 August 1997. Each pair was introduced into a plastic case ($21 \times 15 \times 7$ cm) containing moist filter paper and one current-year shoot of each of the three willow species (*S. sachalinensis*, *S. miyabeana* and *S. integra*). At Ishikari, oviposition of *P. versicolora* was only observed on these three willow species (Ishihara et al. 1999). These plastic cases were placed at 20°C and 16L:8D from 17 to 30 August. All shoots of each willow species were obtained fresh from young clones at Ishikari, and they were replaced by new shoots from different young clones every day. Because host plant clone may affect growth and reproduction of *P. versicolora*, this daily change of clones can diminish the effects of clone-dependent performance. Each time the shoots were replaced, we recorded which willow species had been fed upon. The frequencies at which the leaves of each willow species were fed upon per 13 replacements were nonparametrically compared among the three willow species using a Friedman test, because the distribution of this data was biased from normal due to many zero values. We also conducted multiple pair-wise tests using Wilcoxon signed-ranks tests, and then adjusted significance levels based on a sequential Bonferroni adjustment (Rice 1989).

Larval performance in the laboratory

The development time, larval survivorship, and adult size of *P. versicolora* were compared among the three willow species by raising cohorts of larvae on leaves of each willow species. Twenty-one pairs of the overwintered-generation adults were collected on 28 May 1998 at Ishikari. Each pair was placed in a petri dish containing moist filter paper and *S. sachalinensis* leaves, and was maintained at 22°C and 16L:8D. Leaves were replaced by new ones obtained from a young clone at Ishikari every 2 days. When egg clutches were found on leaves, they were immediately transferred to another petri dish. As soon as the first-instar larvae hatched, a larval cohort was divided into three groups using a split-brood design. Because clutch size might affect larval survivorship (Breden and Wade 1989), each group consisted of 10 larvae. Each group was placed in a petri dish and supplied with leaves of one of the three willow species. The split-brood design, in which each female's offspring were divided equally among the three treatments, ensured genetically matched (family) groups (see Via 1984, 1991). In this way, we established 16 such groups. These petri dishes were maintained at 22°C and 16L:8D. All leaves of each willow species were obtained fresh from a young 5- to 10-year-old clone at Ishikari, and they were replaced by new leaves from a different young clone every 2 days. This daily change of clones can diminish the effects of clone-dependent performance as mentioned above. In our previous study, however, we confirmed that inter-clonal effect of *S. sachalinensis* on larval performance was very small (Hayashi 1998). Probably, the inter-clonal effect of each willow species may be much smaller than the interspecific effect of willows.

When adults emerged, we recorded development time, body size, sex, survivorship, and family group. The development time was determined as the period from hatching to adult emergence. Elytral length was used as an indicator of body size, which was positively correlated with body weight (unpubl. data). Larval survivorship was non-parametrically analyzed using a Friedman test, where family and host plant species were treated as block and treatment, respectively, because the distribution of this data was biased from normal. The individual data on developmental times and body sizes were \log_{10} transformed before analysis to normalize the variance and were analyzed using three-factor mixed model ANOVAs, where host plant species and sex were fixed effects and family was a random effect. Means of individual data were compared among the three willow species using Scheffé's multiple range tests (Scheffé 1959). In the development time, because there was not a significant difference between males and females (Table 1), the pooled data from both sexes were compared among the three willow species.

Table 1 Three-factor mixed model ANOVA for adult willow leaf beetles *Plagiodera versicolora* size and development time (\log_{10} transformed)

Source	Adult size			Developmental time		
	df	SS	F	df	SS	F
Host plant	2	4.332	69.965****	2	585.628	108.550****
Sex	1	31.381	1,410.175****	1	0.665	1.976
Family	15	0.727	3.653****	15	72.140	15.478****
Host plant \times sex	2	0.009	0.219	2	0.900	0.911
Family \times host plant	30	0.929	2.333***	30	80.925	8.682****
Sex \times family	15	0.334	0.677	15	5.051	1.084
Host plant \times sex \times family	30	0.588	1.476	30	14.807	1.588*
Residual	342	4.538		351	109.062	

**** $P < 0.0001$,*** $P < 0.001$, * $P < 0.05$

Cage experiment in the field

The difference in predation pressure on immature larvae of *P. versicolora* between the three willow species (*S. sachalinensis*, *S. miyabeana*, and *S. integra*) was determined in the field site at Ishikari. We selected three similar young clones, which were ca. 150 cm height and 5 years old, from each willow species at the study site on 13 June 1997, and four 1-year shoots were randomly selected from each clone. Each shoot was covered with a mesh-bag, and one female adult collected at this study site was introduced into each mesh-bag. On 25 June 1997 (12 days after introduction), the female adults were removed from the mesh-bags and the numbers of eggs and immature larvae were counted. Approximately one-half of the offspring were first-instar larvae, and the other half were still eggs. All the offspring remaining as eggs hatched within a few days after the removal of adults. The average numbers \pm SDs of total offspring are 54.5 ± 14.6 on *S. sachalinensis*, 50.0 ± 17.4 on *S. integra* and 80.8 ± 18.6 on *S. miyabeana*. We did not adjust the numbers of total offspring because the numbers of offspring per leaf did not vary significantly among willow species (ca. 0.7 offspring per leaf).

After this census, we conducted two treatments per each clone. We removed mesh-bags from three of four 1-year shoots in each clone, but maintained a mesh-bag around the one remaining shoot. In the first treatment, *P. versicolora* larvae were exposed to natural enemies, while in the second treatment the larvae were protected from natural enemies by the mesh-bag. The reason why the number of shoots from which mesh-bags were removed was greater than that on which mesh-bags were maintained was that risk of missing data was higher in the first treatment. After 8 days of these treatments, the numbers of surviving larvae were counted. Because of a lack of significant differences in survivorship among clones within each willow species and small sample sizes, the data from all clones were pooled and total proportions of surviving individuals were compared among willow

species within each treatment using a Tukey-type test for proportions, followed by a chi-square test (Zar 1996). In addition, the total proportions of surviving individuals were also compared between treatments within each willow species using a chi-square test.

Abundance of natural enemies

Because mortality of the first-instar larvae of *P. versicolora* was the key factor determining variation of total mortality from egg to adult emergence among willow clones at the study site (Hayashi 1998), we compared the abundance of natural enemies of the early instar larvae among the three willow species. For eggs and young larvae, the main natural enemies are generalist arthropod predators, such as ants, lady beetles and spiders. We have observed two lady beetle species (*Harmonia axyridis* and *Aiolocaria hexaspilota*) and three ant species (*Camponotus japonicus*, *Lasius hayashi* and *Myrmica jessensis*) as predators feeding on or excluding the eggs and early instar larvae of *P. versicolora* at the study site. However, because these lady beetles were too rare to observe, only the numbers of ants were censused. We randomly selected 11 clones of *S. sachalinensis* and 10 clones each of *S. miyabeana* and *S. integra* at the study site on 2–3 July 1999. We also randomly marked three 1-year shoots from each clone and counted once the number of ants observed on the marked shoots. One variable was derived from each marked 1-year shoot. The numbers of ants observed were compared among willow species using a Kruskal–Wallis test.

Results

Adult preference

There was a significant difference between the frequency of feeding upon each of the three willow species (Friedman

test: $df = 2$, $\chi^2 = 15.08$, $P = 0.0005$). Most feeding was observed on *S. sachalinensis*. The frequency of feeding observed on *S. sachalinensis* was very significantly higher than on other willow species (Fig. 1).

Larval performance in the laboratory

Survivorship from hatching to adult emergence was high on all three willow species and did not significantly vary between them (Kruskal–Wallis test: $n = 48$, $df = 2$, $H = 2.36$, $P = 0.307$). The survivorships (mean \pm SD) were 0.944 ± 0.073 on *S. sachalinensis*, 0.919 ± 0.075 on *S. integra* and 0.956 ± 0.063 on *S. miyabeana*. However, the development time and adult body size (elytral length) varied between willow species. The development time on *S. sachalinensis* was significantly shorter than on *S. integra* but longer than on *S. miyabeana* (Fig. 2). Adult female body size on *S. sachalinensis* was significantly smaller than on *S. miyabeana*, although male body size was not significantly different between the two willow species (Fig. 3). On *S. integra*, body size in both sexes was significantly smaller than on other willow species (Fig. 3). ANOVAs indicated significant host plant and family effects and family \times host plant interactions in the development time and adult size (Table 1). Such effects and

interactions suggest genetic variation in the ability of larvae to use host plants. In addition, a sex effect was indicated by the adult sizes. Among family means, there were no significant correlations except in development time between *S. integra* and *S. miyabeana* (Spearman rank correlation: $r_s = 0.61$, $n = 16$, $P < 0.05$).

Larval performance in the field

For all willow species tested, larval survivorship was significantly higher on caged plants than on exposed plants (*S. Sachalinensis*: $\chi^2 = 148.09$, $P < 0.0001$; *S. integra*: $\chi^2 = 75.62$, $P < 0.0001$; *S. miyabeana*: $\chi^2 = 561.46$, $P < 0.0001$; Fig. 4a vs. b). Larval survivorship within treatments varied significantly among willow species (Fig. 4). The survivorship on exposed *S. miyabeana* was significantly lower than on the other two willow species (Fig. 4b), though that on caged *S. miyabeana* did not differ from that on caged *S. sachalinensis* (Fig. 4a). In contrast, survivorship on exposed *S. integra* was as high as on exposed *S. sachalinensis* (Fig. 4b), though that on caged *S. integra* was significantly lower than that on *S. sachalinensis* (Fig. 4a).

Abundance of natural enemies

There was a marginally significant difference in total number of ants among the three willows species (Kruskal–

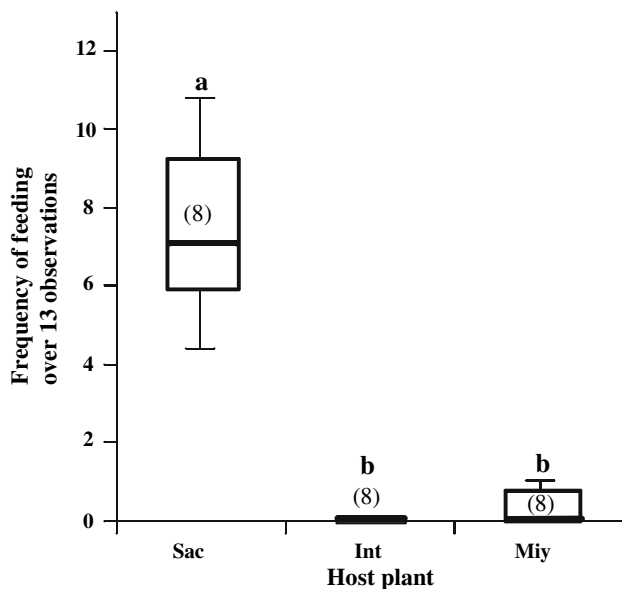


Fig. 1 Frequency of adult willow leaf beetles *Plagioder versicolora* feeding observed on leaves of the three willow species (Sac = *Salix sachalinensis*; Int = *S. integra*; Miy = *S. miyabeana*) over 13 observations carried out from 17 to 30 August 1997 at 20°C, 16L–8D, in a choice test. The box gives upper and lower quartiles, the horizontal bar shows the median, and the whiskers extend to the 10th and 90th percentiles. Comparisons with different letters indicate significant differences (multiple pair-wise test by Wilcoxon signed-ranks tests using a sequential Bonferroni adjustment, $P < 0.05$)

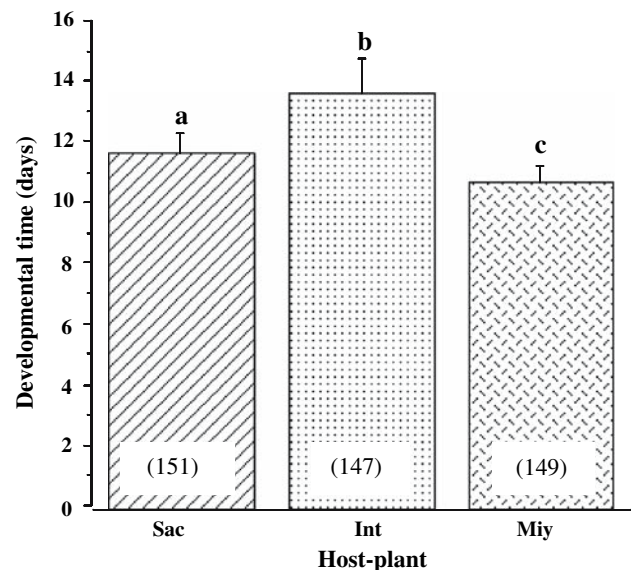
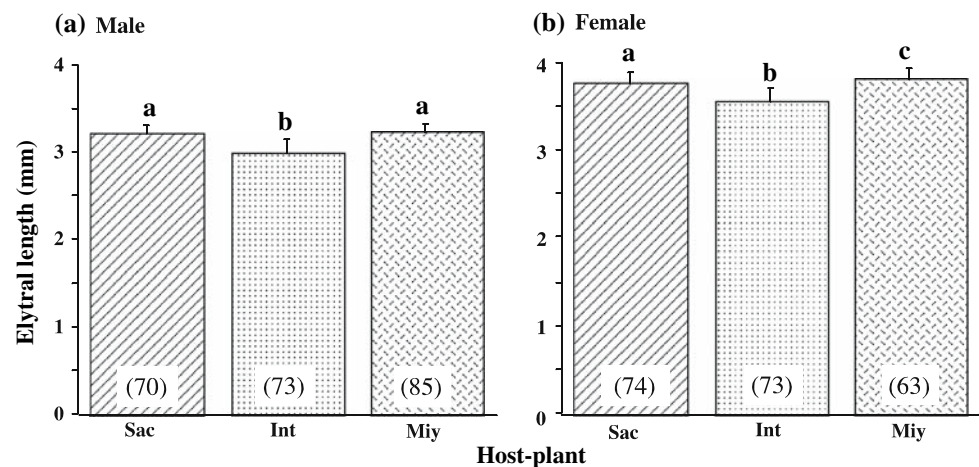


Fig. 2 Development time from hatching to adult emergence on the three willow species (Sac = *S. sachalinensis*; Int = *S. integra*; Miy = *S. miyabeana*) at 22 °C, 16L:8D. Bars represent mean + SD. Comparisons with different letters indicate significant differences (Scheffé's multiple range test: $P < 0.05$). Sample sizes are given in parentheses

Fig. 3 Effects of larval food on adult body size of **a** males and **b** females. Larvae were bred on the three willow species (Sac = *S. sachalinensis*; Int = *S. integra*; Miy = *S. miyabeana*) at 22°C, 16L:8D. Bars represent mean + SD. Comparisons with different letters indicate significant differences (Scheffé's multiple range test: $P < 0.05$). Sample sizes are given in parentheses



Wallis test: $df = 2$, $H = 5.67$, $P = 0.059$). The numbers (mean \pm SD) of ants per 100 leaves on *S. miyabeana*, *S. sachalinensis* and *S. integra* were 4.9 ± 14.3 , 0 ± 0 and 0.1 ± 0.1 , respectively.

Discussion

Adult preference

Plagiodera versicolora adults strongly preferred to feed on *S. sachalinensis* over *S. integra* and *S. miyabeana*. This strong feeding preference for *S. sachalinensis* can explain why most *P. versicolora* adults are found on *S. sachalinensis* at the study site (Ishihara et al. 1999). We randomly selected 11 clones of *S. miyabeana*, 22 clones of *S. sachalinensis* and 11 clones of *S. integra* and marked 10–46, 1-year shoots of each clone. Adults and egg clutches on the marked 1-year shoots were counted at least once every several days from 17 May to 17 July 1999. Throughout the

season, greater number of adults and most eggs were found on *S. sachalinensis* even though ovipositing females could move freely among willow species, and the abundance of *S. sachalinensis* was half that of the most abundant willow species, *S. miyabeana*, at the study site (Ishihara et al. 1999). This tendency has been observed in other populations of *P. versicolora* (unpubl. data). At Kohya-cho (Wakayama, Japan), *S. ericarpa*, on which greater number of adults was observed, had more eggs than other willow species. These evidence suggest that feeding preference coincides with oviposition preference in *P. versicolora*, although we have not confirmed this experimentally.

Bottom-up effects of host plant quality on larval performance

There were significant differences in all measures of larval performance, except for survivorship, among the three willow species. In both development time and adult body

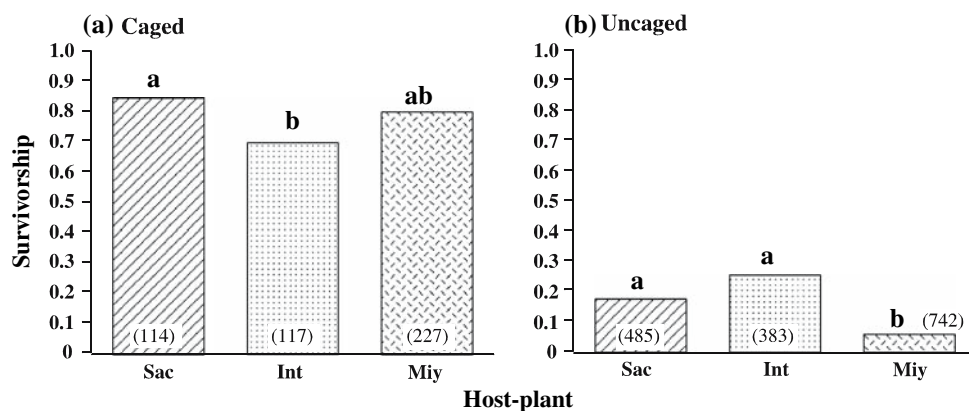


Fig. 4 Survivorships of first-instar larvae on the three willow species (Sac = *S. sachalinensis*; Int = *S. integra*; Miy = *S. miyabeana*), **a** from which predators were excluded by mesh-bags and **b** those which were exposed to predators for 8 days (25 June–3 July 1997) at the field site

(Ishikari, Hokkaido, Japan). Comparisons with different letters indicate significant differences (Tukey-type test for proportions: $P < 0.05$). Sample sizes are given in parentheses

size, performance on *S. integra* was lower than on *S. sachalinensis* and *S. miyabeana*. In many herbivorous insects, adult body size is strongly correlated with potential fecundity (e.g., Leather 1988; Carter et al. 1991; Klingenberg and Spence 1997; but see Ohgushi 1996). In *P. versicolora*, the small body size achieved on *S. integra* may result in a decrease in fecundity. In addition, according to the slow growth/high mortality hypothesis (Feeny 1976; Clancy and Price 1987; Häggström and Larsson 1995), larvae on *S. integra* may suffer higher enemy-caused mortality owing to the longer period spent in vulnerable larval stages compared with ones on *S. sachalinensis* and *S. miyabeana*. However, in this case such effect should be weak because the difference in developmental time between *S. integra* and *S. miyabeana* was only 3 days.

The predator exclusion experiment in the field showed that the survivorship of immature larvae on *S. integra* was significantly lower than on *S. sachalinensis* (Fig. 4a). However, survivorship from hatching to adult emergence in the laboratory did not significantly vary among willow species, and most larvae on all willow species were able to survive until the adult stage. Even in survivorship of the early instar larvae, there was no significant difference between willow species. This difference in survivorship between laboratory and field experiments may be caused by the difference in induced defenses against *P. versicolora* between the live plants in the field and cut shoots taken to the laboratory. Although the cut shoots of *S. integra* might not show an induced defense, the plants in the field may show a stronger induced defense than *S. sachalinensis*. Induced defense in *S. integra* has also been suggested by data from other populations of *P. versicolora*. When *S. integra* was severely damaged by herbivores, the larval performance of *P. versicolora* on *S. integra* after the damage was lower than that before the damage (H. Yoshikawa, personal communication).

Thus, larval performance on *S. integra* was lower than on *S. sachalinensis* and *S. miyabeana*. However, the absolute difference in larval performance between *S. integra* and other willows may not be large. The most important cause of the lack of preference for *S. integra* might be the small leaf size of *S. integra*. Leaf area of *S. integra* is less than half of that of *S. sachalinensis* and *S. miyabeana*. Larvae raised on *S. integra* must move more frequently between leaves for feeding over the course of development than those raised on other willows. This frequent movement should be costly for larvae because this might not only prolong development time but also increase the risk of predation at mature larval stages when larval feeding becomes most active.

Therefore, the adult preference for *S. sachalinensis* over *S. integra* is thought to be adaptive for *P. versicolora*.

However, although the larval performance on *S. miyabeana* was same or better than that on *S. sachalinensis*, and *S. miyabeana* is the most abundant willow species at the study site, adult preference for *S. miyabeana* was much weaker than for *S. sachalinensis*. At the study site, only a few adults and eggs were found on *S. miyabeana* (Ishihara et al. 1999). It is unclear why *S. miyabeana* is not preferred by ovipositing adults in spite of its qualitative and quantitative advantages.

Top-down effects by natural enemies on larval performance

The enemy-free space hypothesis predicts that variability in the relative safety of alternative niches will influence the evolution of herbivore niche preferences (Jeffries and Lawton 1984; Berdegue et al. 1996). The cage experiment supports the enemy-free space hypothesis, because the preference of *P. versicolora* for *S. sachalinensis* has been favored by the relative safety of *S. sachalinensis* compared to *S. miyabeana*. In this experiment, we were able to use only three clones of each willow species. This small sample size may not be adequate if there were strong plant genotype effects on predation pressure. However, the magnitudes of the effects of predation were so large that we were convinced that predation pressure was adequately demonstrated. The predation pressure on early immatures on *S. miyabeana* was significantly higher than that on other willow species. Because the mortality of first-instar larvae is the key factor determining in variation of total mortality from egg to adult emergence among willow clones at the study site (Hayashi 1998), the difference in predation pressure on first-instar larvae among willow species may be an important factor for *P. versicolora* to determine its oviposition preference. Therefore, *P. versicolora* is likely to have responded to these circumstances by evolving a strong preference for *S. sachalinensis* despite the nutritional suitability of *S. miyabeana*. In the predator exclusion and laboratory experiments, the larval performance of *P. versicolora* on *S. miyabeana* was the same or even higher than on its preferred host, *S. sachalinensis*.

The difference in survivorship of early immatures between caged and uncaged shoots may be partly caused by the better microclimate inside the cage. However, the effect of microclimate on this difference should be weaker than the impact of predation, because we frequently observed that eggs and early instar larvae on the exposed shoots were eaten or removed by generalist arthropods, such as ants, lady beetles and spiders (M. Ishihara, personal observation). In addition, we could identify the strong predation pressure from such predators on eggs on the

exposed shoots by abundant stains and egg shells remaining on leaves. These arthropods are the main predators of leaf beetle larvae. When *P. versicolora* larvae are attacked by these predators, they release defensive compounds from specialized exocrine glands in defense (Meinwald et al. 1977; Sugawara et al. 1979; Matsuda and Sugawara 1980; Pasteels et al. 1990). Although we observed that the secretion released by mature larvae repelled such predators in the laboratory (M. Ishihara, personal observation), eggs and early instar larvae may not have the ability to defend against the predators.

The present study indicates that, even in the herbivorous insects that do not use plant chemicals as their defense against natural enemies, the role of enemy-free space can be important in determining host utilization patterns. However, a number of studies on the relationship between adult host preference and offspring performance have been conducted in the absence of natural enemies under laboratory conditions (see Thompson and Pellmyr 1991), which have ignored the important role of top-down effects by natural enemies on the host utilization pattern of herbivorous insects. Yamaga and Ohgushi (1999) and Heard et al. (2006) argued that the spatial and temporal variability of natural enemies is a key component in alternating the preference–performance linkage that is predicted by host plant species quality. In general, top-down effects by natural enemies on herbivores should vary among host plants (Thompson 1988b). Both top-down and bottom-up effects should be key factors in the determination and evolution of the host utilization patterns of herbivores. Thus, more studies are needed to fully understand the importance of top-down effects by natural enemies on the host utilization patterns of herbivorous insects.

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