Adaptive change and conservatism in host specificity in two local populations of the thistle-feeding ladybird beetle *Epilachna niponica*

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Abstract

We investigated geographic differences in the host specificity of *Epilachna niponica* Lewis (Coleoptera: Coccinellidae). The Yuwaku population feeds mainly on *Cirsium matsumurae* Nakai (Asteraceae) and secondarily on *Cirsrium kagamontanum* Nakai. The Asiu population, located 150 km away from the Yuwaku, feeds exclusively on *C. ashiuense* Yokoyama et T. Shimizu. Under laboratory conditions, we examined the differences between the two populations in adult feeding acceptance, adult feeding preference, and larval performance, using several closely related thistle species and varieties, including their native hosts. In the Asiu population, adult beetles clearly avoided the host of the Yuwaku population, *C. kagamontanum*, and no larvae were able to complete their development, whereas in the Yuwaku population, adults accepted and even preferred it to some other thistle species, and about 10% of first instar larvae became adults. This indicates that the Yuwaku population evolved its feeding preference and physiological adaptation to *C. kagamontanum* through a utilization of this low-ranked host under natural conditions. Apart from *C. kagamontanum*, the two populations showed a similar host susceptibility pattern, indicating that this ladybird beetle has a conserved hierarchy in feeding preference and growth performance. We also observed adult leaf choice behavior when given different thistle species, and found that difference in biting rate after palpation determined the leaf areas consumed, implying that factors on the leaf surface played an important role in the choice.

Introduction

Many species of phytophagous insects show differentiation amongst populations in their patterns of host plant utilization (Singer, 1971; Fox & Morrow, 1981; Hsiao, 1982; Scriber, 1983; Zwölfer & Romstöck-Völkl, 1991). Observed differences in local host-use patterns may simply reflect differences in host-plant abundance or quality in each habitat, or may be the result of adaptations to local conditions (Fox & Morrow, 1981; Jaenike, 1990; Mayhew, 1997). They may also be attributable to the geographic variation of the plants (Fritz & Simms, 1992). Interpopulation divergence in host use has been investigated in experimental studies on many insect species, by offering several potential host plants to insects from local populations that depend on different host plants under natural conditions. However, results have been variable. In some cases, the preference hierarchies of potential host plants varied, reflecting native host plants in their natural habitats, in populations of the nymphaline butterfly, *Euphydryas editha* (Singer, 1971, 1983; Thomas et al., 1987; Singer et al., 1989, 1992), the tiger swallowtail, *Papilio glaucus* (Scriber, 1983), the Colorado potato beetle, *Leptinotarsa decemlineata* (Hsiao, 1978; Hare & Kennedy, 1986), and insects feeding on thistle flower-heads such as the weevils *Larinus sturnus* and *Larinus jaceae*, and the fruit fly *Tephritis conura* (Zwölfer & Romstöck-Völkl, 1991). In other cases, populations of *Drosophila tripunctata* (Jaenike, 1989) and the cowpea weevil, *Callosobruchus maculatus*

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(Wasserman, 1986), did not differ geographically in their preference rankings in spite of the difference in the actual hosts. These cases imply that the preference hierarchy is 'evolutionarily conservative' (Thompson, 1993). Finally, in some cases such as the anise swallowtail butterfly, *Papilio zelicaon* (Thompson, 1993), the overall preference hierarchy was similar in local populations, but slight differences increasing the preference to a local host were observed, this being intermediate between the first and second cases.

Epilachna niponica Lewis (Coleoptera: Coccinellidae), a specialist herbivore of wild thistles (genus *Cirsium*), is a member of a group of closely related ladybird beetles called the *Epilachna vigintioctomaculata* species complex, which exhibits remarkable inter- and intraspecific variations in external morphology and food plants (Katakura, 1981, 1997; Hoshikawa, 1983). The geographic structure of host specificity in *E. niponica* is of particular interest, because its host-use pattern varies considerably among local populations. Earlier studies have indicated that *E. niponica* shows extensive geographic variation in many characteristics such as elytral shape (Katakura, 1981; Tohyama, 1997), allozyme (Tanaka, 1997), and mt-DNA polymorphism (Nagata, 2002). Thistles, the host plant, are also highly diversified in Japan, and are taxonomically complex because many species have great morphological variation among local populations, and because they hybridize easily (Kitamura, 1937). In central Honshu, the main island of Japan, the geographical distribution of each thistle species is restricted and usually replaced successively by a related species (Kitamura, 1937; Kadota, 1995). Therefore, each local population of *E. niponica* generally depends on only a few thistle species (M. Yamashita, unpubl.). Such a host-use pattern of *E. niponica* provides a good natural arena for studying the evolution of host specificity in phytophagous insects.

We investigated differences in the host plant specificity of *E. niponica* from two geographically distant populations that vary in their host plant utilization in the field. We expected to find that the differences in host plant availability and utilization under natural conditions between the two populations would lead to a divergence in adult feeding preference and larval performance. We tested these expectations in the laboratory using three experimental set-ups: (1) several thistle species were presented singly to adult females (feeding acceptance experiments), (2) several thistle species were presented simultaneously to adult females (feeding preference experiments), and (3) larval rearing (larval performance experiments). Furthermore, we investigated whether the two populations showed differences in post-alighting feeding behavior (palpating and biting) on different host plants by a direct observation of adult behavior in the feeding preference experiments.

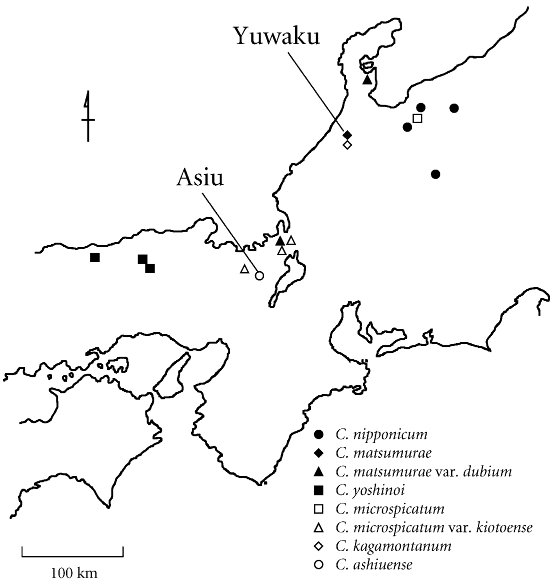

Figure 1 Sampling localities of *Epilachna niponica* (Yuwaku and Asiu) and/or thistles used in the experiments. Both *Cirsium matsumurae* and *C. kagamontanum* were collected from Yuwaku.

Materials and methods

Insects

Two geographically distant *E. niponica* populations were investigated (Figure 1). The Yuwaku population in Kanazawa, Ishikawa Prefecture, has both *C. matsumurae* and *C. kagamontanum* in its habitat, but it is found primarily on the former species and only occasionally on the latter (Kaihara et al., 1997; Koji & Nakamura, 2002). The Asiu population in the northern part of Kyoto Prefecture (about 150 km southwest of the Yuwaku population), only has *C. ashiuense* available, and occurs exclusively on this plant (Nakamura & Ohgushi, 1979). The Yuwaku and Asiu populations show similar life cycles: overwintered adults emerge on the thistles from April to June, and new adults appear from July to October and enter diapause by late fall. Most overwintered adults die by summer, but some enter a second diapause (Nakamura & Ohgushi, 1979; Koji & Nakamura, 2002). Compared with the Asiu population, the Yuwaku population is characterized by beetles with larger body size and a shorter life span of overwintered adults (Nakamura, 1983; Koji & Nakamura, 2002). The Yuwaku population often reached a density so great it depleted local food sources, while the Asiu population remained at a relatively constant low density below the food depletion level. The proportion of adults that overwintered twice was also higher in Asiu than in Yuwaku (Nakamura & Ohgushi, 1979).

We collected post-hibernating adults of *E. niponica* in the spring of 1994 from both populations and kept them in the

Table 1 Host-use pattern of two *Epilachna niponica* populations for different thistle species and varieties

		Population[a]	
Subsection	Species/varieties	Yuwaku	Asiu
Nipponocirsium	*Cirsium matsumurae*	M	NM
	Cirsium matsumurae var. *dubium*	NM	NM
	Cirsium nipponicum	NM	NM
	Cirsium nipponicum var. *yoshinoi*	NM	NM
Tubulosae	*Cirsium microspicatum*	NS	NS
	Cirsium microspicatum var. *kiotoense*	NS	NS
Reflexae	*Cirsium kagamontanum*	S	NS
	Cirsium ashiuense	NM	M

[a] M: Main host species; S: subsidiary host species; NM: novel species that are utilized as main hosts by other populations; NS: novel species that are utilized as subsidiary hosts by other populations.

laboratory under a controlled regime of L14:D10 at 20 °C. All experiments were conducted between April and August of 1994.

Plants

We used five species and three varieties of thistle, distributed from central to west Honshu, to examine their quality as diets of *E. niponica*: two species and two varieties in subsection Nipponocirsium (*C. matsumurae*, *C. matsumurae* Nakai var. *dubium*, *C. nipponicum* (Maxim.) Makino, and *C. nipponicum* (Maxim.) Makino var. *yoshinoi*), one species and one variety in subsection Tubulosae (*C. microspicatum* Nakai and *C. microspicatum* Nakai var. *kiotoense*), and two species in subsection Reflexae (*C. kagamontanum*, *C. ashiuense*) (Kadota, 1995, Table 1). We classified them into four categories: (1) main host plant, on which all life stages of *E. niponica* can be found (indicated by 'M'), (2) subsidiary host plant, occasionally eaten by adults or larvae in the presence of the main host species (S), (3) novel main host, novel to the Yuwaku and Asiu populations, but utilized as the main hosts by other beetle populations (NM), and (4) novel subsidiary host, not encountered by either of the populations studied, but are utilized as subsidiary host plants by other beetle populations (NS).

We collected *C. matsumurae* and *C. kagamontanum* leaves at Yuwaku. We collected plants of the other six thistle species and varieties from various localities in central Honshu, and potted or transplanted them to the Botanic Garden of Kanazawa University (Figure 1). We kept leaves in plastic bags at 5 °C, and used them for the experiments (see below) within 2 days of sampling.

Feeding acceptance of adults

We examined the feeding acceptance of adult beetles to eight thistle species/varieties using no-choice design tests. For each thistle species, we punched out leaf disks (3.5 cm in diameter) using a circular punch press. We placed individual female beetles on a leaf disk of a particular test thistle species in a transparent plastic cup (11 cm diameter, 4.5 cm depth) with the bottom covered with moist filter paper. We examined 3–6 plants for each thistle species with 39–67 beetles. After 24 h, we measured the leaf area consumed to the nearest 1 mm^2 using a transparent grid. Beetles were repeatedly assayed for different thistle species: eight rounds for the Yuwaku population, and four rounds for two groups of the Asiu population. Between the two successive experiments, the beetles were maintained with 10% sucrose solution for more than 24 h in order to minimize the influence of the preceding feeding tests. Experiments were carried out under a controlled regime of L14:D10 at 20 °C.

Feeding preference of adults

Preference ranking. The feeding preference of adult beetles was examined by multiple design choice tests with four thistle species/varieties: *C. matsumurae* (M for Yuwaku, NM for Asiu, see Table 1), *C. matsumurae* var. *dubium* (NM for both populations), *C. kagamontanum* (S for Yuwaku, NS for Asiu), and *C. ashiuense* (NM for Yuwaku, M for Asiu). The preference rank for each beetle was determined with the following procedure:

(1) 4-choice test: leaf discs were cut into quarters and placed in glass Petri dishes (21 cm in diameter, 4.5 cm depth) with the bottom covered with moist filter paper. A set consisting of pieces from each of the four thistle species was placed in a circle and offered to each female beetle. After 2 h, we removed the leaf pieces and measured the areas consumed. (2) 3-choice test: the experiment was continued with the same adults in the same way with a new set of three leaf pieces from the three thistle species except the one most preferred (most consumed) in the 4-choice

test. (3) 2-choice test: the experiment was continued with a new set of two leaf pieces from the two thistle species except the one most preferred in the 3-choice test.

Based on the order of removal of the thistle species in the successive tests, the preference ranking from 1 (not removed and least eaten) to 4 (firstly removed) was determined. In case of ties (i.e., leaf pieces were eaten equally) or no pieces at all were eaten, we repeated the test with the same set-up. If ties were repeated in two successive tests, those plants were given the mean of their combined ranks in the second test and removed. Individuals that did not eat any leaf disks for two successive tests were excluded from the analysis. A total of 34 and 30 females were tested for the Yuwaku and Asiu populations, respectively. Assays were carried out under uncontrolled conditions in a room at a temperature of approximately 23 °C.

Behavioral observation. For each assay, the behavior of individual beetles was recorded by three people, each of whom observed different groups of up to 12 beetles simultaneously. Since the beetles were sluggish and at any given time several beetles were resting, it was possible to follow the activities of all experimental insects simultaneously without any loss of information. Food choice behavior was categorized into two patterns. First, 'palpating' was defined by a series of behavioral events of walking on a leaf disc, repetitive contact with the leaf surface by the maxillary palpi, and antennal waving. In this process, sensory stimuli are probably received by sensilla on the antennae, maxillary palps, and/or labial palps (Harrison, 1987). Second, 'biting' following palpating involved biting of the leaf surface with the mandibles. After biting, beetles subsequently fed on the leaf tissue or immediately terminated biting and began to walk around. The number of occurrences of palpating and biting on each leaf piece was recorded for each individual. Although either 34 or 30 individual beetles were examined in the choice tests for each population, the assays were repeated with the same beetles that did not show a preference, causing sample size variation among the experiments (Figures 3 and 4).

Larval performance

The performance of the larvae on four thistle species/varieties (*C. matsumurae*, *C. matsumurae* var. *dubium*, *C. kagamontanum*, and *C. ashiuense*) was examined by rearing them from hatching to adult emergence. Newly hatched larvae were obtained from eggs produced by beetles used in the above experiments. To minimize genetic differences of the beetles among rearings, we divided hatched larvae from one egg mass into two or three groups, and reared each group with a sufficient amount of fresh leaves in a transparent styrene box (8.0 × 15.3 × 3.0 cm), the bottom of which was covered with moist filter paper. Survival rate and development time (days) from hatching to adult emergence were recorded. The pronotum width of newly emerged adults was also recorded as a measure of body size. Rearing was carried out under a controlled regime of L14:D10 at 20 °C.

Results

Feeding acceptance of adults

Mean areas consumed varied significantly between the Yuwaku and Asiu populations and among the thistle species (Figure 2, Table 2). The interaction between population and host species was also significant, indicating that the two populations differed in their host acceptance patterns. Consumed areas were often significantly different in pair-wise comparisons (Figure 2). For both populations, the consumed leaf area was large for *C. matsumurae* (main host plant of the Yuwaku population) (mean ± SE: 214.7 ± 16.4 mm^2 and 253.4 ± 24.6 mm^2 for the Yuwaku and Asiu populations, respectively), and small for *C. ashiuense* (main host plant of the Asiu population) (26.5 ± 7.1 mm^2 and 45.7 ± 7.6 mm^2

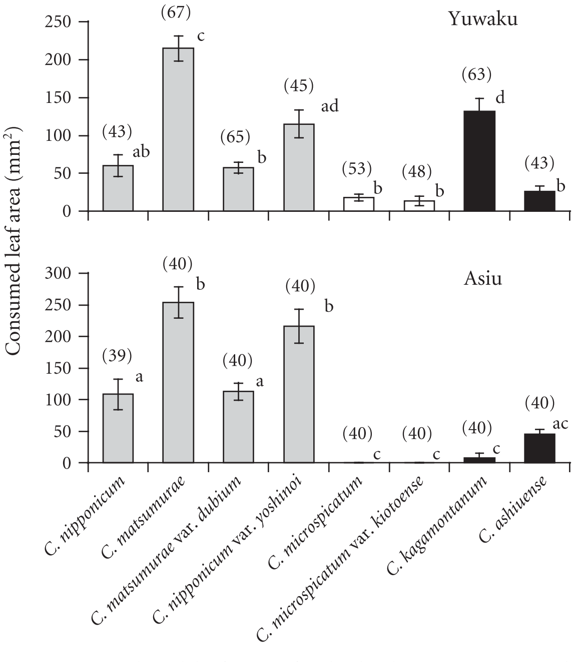

Figure 2 Mean (± SE) leaf areas of eight thistle species consumed by overwintered adults of *Epilachna niponica* from the Yuwaku (upper) and Asiu (lower) populations in no-choice tests. Grey, open, and black columns represent thistle species of the subsection Nipponocirsium, Tubulosae, and Reflexae, respectively. Number of females used is shown in parentheses. Bars with same letters are not significantly different (P>0.05, Tukey–Kramer tests after log transformation).

Table 2 Two-way ANOVA of mean consumed areas obtained by no-choice tests

Source	d.f.	MS	F	P
Population	1	3.197	6.224	0.013
Host species	7	44.237	86.118	<0.001
Population × host species	7	12.934	25.179	<0.001
Error	730	0.514		

Data were log-transformed before analysis.

for the Yuwaku and Asiu populations, respectively). In contrast, acceptance of *C. kagamontanum* (the subsidiary host in Yuwaku) was remarkably different between the two populations: it was high for the Yuwaku (132.1 ± 16.6 mm^2) but low for the Asiu population (7.9 ± 7.1 mm^2).

Apart from *C. kagamontanum*, the ranking in consumed areas of the thistle species was similar for the two populations: acceptance of the thistle species of the subsection Nipponocirsium was higher than those of the subsection Tubelosae (Scheffé method for multiple comparison; Yuwaku: F = 9.97, d.f. = 7, P<0.01; Asiu: F = 20.66, d.f. = 7, P<0.01). Acceptance for the subsection Reflexae was high in the Yuwaku population (Scheffé method; Reflexae vs. Nipponocirsium: F = 1.16, d.f. = 7, P>0.05; Reflexae vs. Tubelosae: F = 3.24, d.f. = 7, P<0.01) but low in the Asiu population population (Scheffé method; Reflexae vs. Nipponocirsium: F = 14.75, d.f. = 7, P<0.01; Reflexae vs. Tubelosae: F = 0.37, d.f. = 7, P >0.05).

Feeding preference of adults

Preference ranking. The preference ranking was determined for 31 and 23 beetles from the Yuwaku and Asiu populations, respectively, and the result was highly consistent among the beetles in each population (Kendall's coefficient concordance: Q = 65.84 and 43.32 for Yuwaku and Asiu, respectively, d.f. = 3, P<0.001). For the Yuwaku population, the mean rank of thistle species was in the following descending order: *C. matsumurae* var. *dubium* (3.44)>*C. matsumurae* (3.19)>*C. kagamontanum* (1.94)>*C. ashiuense* (1.44). For the Asiu population, it was: *C. matsumurae* var. *dubium* (3.33)>*C. matsumurae* (2.83)>*C. ashiuense* (2.33)>*C. kagamontanum* (1.50).

Behavioral patterns determining the differential preference of the beetles among the thistle species. Figures 3 and 4 show palpating frequency, biting rate (per cent of biting after palpating) and consumed leaf area for Yuwaku and Asiu populations.

Yuwaku population: The 4-choice tests indicated statistically significant differences in the palpating frequency [MANOVA (Manly, 1993): $F_{3,51}$ = 8.622, P<0.001] and consumed leaf areas (MANOVA: $F_{3,51}$ = 64.946, P<0.001) (Figure 3a). Adults preferred the thistle species/varieties of the subsection Nipponocirsium (*C. matsumurae* and *C. matsumurae* var. *dubium*) to those of the subsection Reflexae (*C. kagamontanum* and *C. ashiuense*). *Cirsium kagamontanum* was preferred to *C. ashiuense*. The mean consumed leaf area was strikingly different between *C. kagamontanum* (2.5 ± 0.8 mm^2) and *C. ashiuense* (not consumed at all), which resulted from the difference in biting rate (26.1% for *C. kagamontanum* and 1.7% for *C. ashiuense*), rather than in palpating frequency (1.3 ± 0.2 for *C. kagamontanum* and 1.7 ± 0.2 for *C. ashiuense*) (Figure 3a). The 3-choice (Figure 3b,c) and 2-choice tests (Figure 3d), gave similar results. In the 3-choice test with *C. matsumurae*, *C. kagamontanum*, and *C. ashiuense* (Figure 3b), the palpating frequency and consumed leaf areas were significantly different (MANOVA: palpating

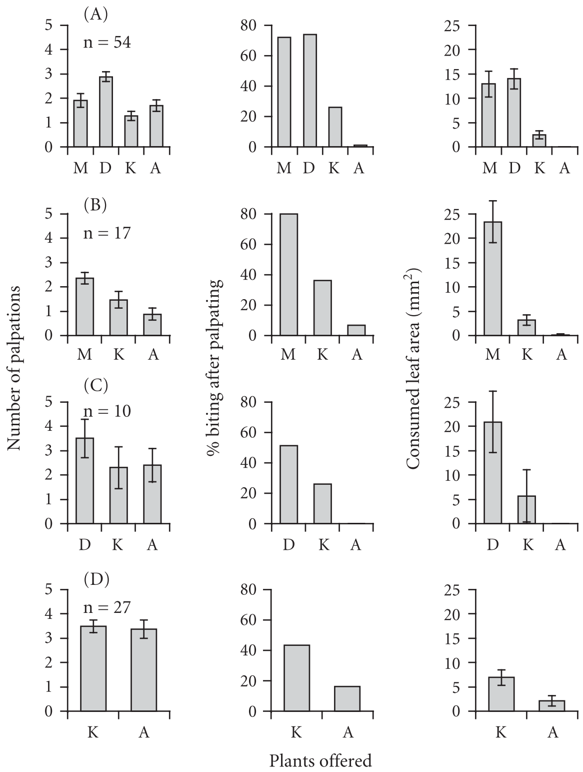

Figure 3 Response of adult females of the Yuwaku population to different thistle species (M, *Cirsium matsumurae*; K, *C. kagamontanum*; A, *C. ashiuense*; D, *C. matsumurae* var. *dubium*) in 4-choice experiments (A), 3-choice experiments with M, K, A (B) and K, A, D (C), and 2-choice experiments with K, A (D). Left: Mean number of palpations. Center: percentage biting after palpation. Right: Mean leaf area consumed. Vertical lines represent ± SE.

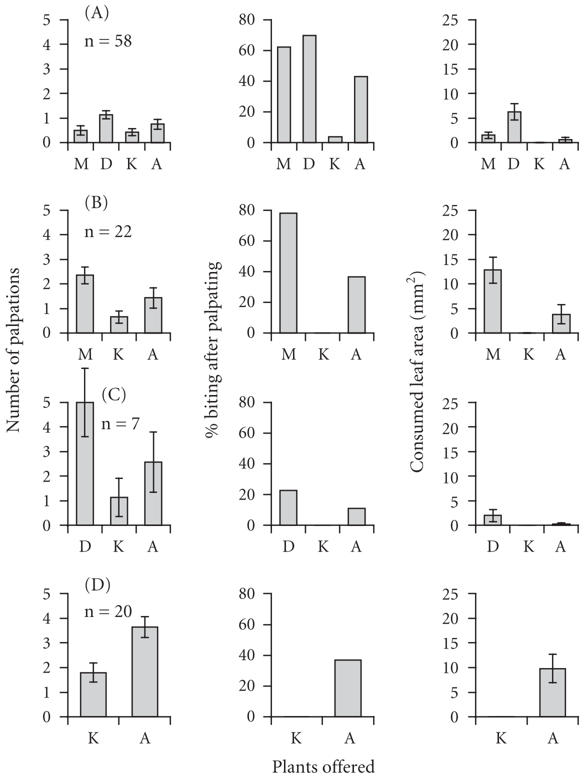

Figure 4 Response of adult females of the Asiu population to different thistle species (M, *Cirsium matsumurae*; K, *C. kagamontanum*; A, *C. ashiuense*; and D, *C. matsumurae* var. *dubium*) in 4-choice experiments (A), 3-choice experiments with M, K, A (B) and K, A, D (C), and 2-choice experiments with K, A (D). Left: Mean number of palpations. Center: percentage biting after palpating. Right: Mean leaf area consumed. Vertical lines represent ± SE.

frequency, $F_{2,15} = 11.888$, $P<0.001$; consumed leaf area, $F_{2,15} = 39.967$, $P<0.001$), and the preference ranking was: *C. matsumurae*>*C. kagamontanum*>*C. ashiuense*. In the experiment with *C. matsumurae* var. *dubium*, *C. kagamontanum* and *C. ashiuense* (Figure 3c), all individuals fed only on *C. matsumurae* var. *dubium* except the one beetle that fed on *C. kagamontanum*, although the difference in palpating frequency was insignificant (MANOVA: $F_{2,8} = 0.924$, $P = 0.436$). In the 2-choice tests with low-ranked *C. kagamontanum* and *C. ashiuense* (Figure 3d), the biting rate was higher for *C. kagamontanum* (43.6%) than for *C. ashiuense* (16.5%), and the consumed leaf area was also larger for *C. kagamontanum* (MANOVA: $F_{1,26} = 9.184$, $P = 0.005$), although there was no difference in the palpating frequency between the two thistle species (MANOVA: $F_{1,26} = 0.114$, $P = 0.738$).

The Asiu population: 4-choice tests showed statistically significant preferences in palpating frequency (MANOVA: $F_{3,56} = 4.889$, $P<0.004$) and consumed leaf areas (MANOVA: $F_{3,56} = 12.237$, $P<0.001$) (Figure 4a). The Asiu population also preferred *C. matsumurae* and *C. matsumurae* var. *dubium* to *C. kagamontanum* and *C. ashiuense*, as did the Yuwaku population. However, between the two less preferred thistle species, the Asiu beetles preferred *C. ashiuense* (mean consumed leaf area: 0.6 ± 0.5 mm^2) to *C. kagamontanum* (not consumed) (Figure 4a).

Results of the 4-choice test were consistent with the 3-choice (Figure 4b,c) and 2-choice tests (Figure 4d) as follows: In 3-choice tests with *C. matsumurae*, *C. kagamontanum*, and *C. ashiuense* (Figure 4b), the palpating frequency (MANOVA: $F_{2,20} = 6.668$, $P = 0.006$) and consumed leaf area (MANOVA: Asiu; $F_{2,20} = 24.073$, $P<0.001$) were significantly different. Adults preferred *C. matsumurae* to *C. ashiuense*, and they did not bite *C. kagamontanum* at all. In 3-choice tests with *C. matsumurae* var. *dubium*, *C. kagamontanum*, and *C. ashiuense* (Figure 4c), the preference order was: *C. matsumurae* var. *dubium*>*C. ashiuense*>*C. kagamontanum* (not consumed at all), although palpating frequency was not significantly different between them (MANOVA: $F_{2,5} = 2.968$, $P = 0.141$), and consumed leaf areas were small (0–2.0 mm^2) and not statistically different (MANOVA: $F_{2,6} = 2.712$, $P = 0.145$). In 2-choice experiments with *C. kagamontanum* and *C. ashiuense* (Figure 4d), the Asiu population preferred *C. ashiuense* to *C. kagamontanum*, according to the palpating frequency (MANOVA: $F_{1,19} = 24.100$, $P<0.001$), biting rate (37.0% and 0%, respectively), and consumed leaf area (MANOVA: $F_{1,19} = 21.527$, $P<0.001$).

Larval performance

Table 3 summarizes the results of the rearing experiments. For both populations, all indices of growth performance except male pronotum width of the Yuwaku population were significantly different among thistle species/varieties. For the Yuwaku population, *C. kagamontanum* was the poorest host plant, with a low eclosion rate (9.6%), prolonged growth (44.6 ± 0.6 days), and only five beetles completing development, all with small body size (3.20 ± 0.12 mm for females, 3.4 mm for one male). There was no significant difference in these traits among the other three thistle species. For the Asiu population, on the other hand, all larvae reared on *C. kagamontanum* died before eclosion. *Cirsium ashiuense* (the native host plant in Asiu) and *C. matsumurae* var. *dubium* were the most suitable for larval growth (Table 3). Larvae reared on *C. matsumurae*, the species most preferred by adults, showed a low eclosion rate (27.5%), prolonged development (40.09 ± 1.15 days) and small body size (3.17 ± 0.04 mm and 3.04 ± 0.03 mm for females and males, respectively).

Table 3 Developmental traits (means ± SE) of two *Epilachna niponica* populations reared on four thistle species/varieties. Number of beetles in parentheses

		Eclosion	Developmental	Pronotum width (mm)†	
Population	Plant offered	rate*	duration (days)†	Female	Male
Yuwaku	*C. matsumurae*	68.8^a (112)	38.75 ± 0.53^a (77)	3.49 ± 0.03^a (43)	3.28 ± 0.03^a (34)
	C. kagamontanum	9.6^b (52)	44.60 ± 0.60^b (5)	3.20 ± 0.12^b (4)	3.35 (1)
	C. ashiuense	65.1^a (43)	38.89 ± 0.73^a (28)	3.44 ± 0.07^{ab} (12)	3.31 ± 0.03^a (16)
	C. matsumurae var. *dubium*	54.8^a (62)	40.26 ± 0.56^{ab} (34)	3.56 ± 0.03^a (18)	3.36 ± 0.03^a (16)
Asiu	*C. matsumurae*	27.5^a (120)	40.09 ± 1.15^a (33)	3.17 ± 0.04^a (16)	3.04 ± 0.03^a (17)
	C. kagamontanum	0.0^b (139)	– (0)	– (0)	– (0)
	C. ashiuense	63.5^c (170)	34.96 ± 0.36^b (108)	3.36 ± 0.02^b (73)	3.16 ± 0.03^b (35)
	C. matsumurae var. *dubium*	67.9^c (109)	37.56 ± 0.36^c (74)	3.36 ± 0.02^b (44)	3.14 ± 0.02^{ab} (29)

Figures with different letters are significantly different ($P<0.05$).

*Analyzed by χ^2 tests with sequential Shaffer's correction of significance level.

†Analyzed by Tukey–Kramer test. Data were log-transformed before analyses.

Discussion

This study showed that beetles from the Yuwaku and Asiu populations were able to recognize the differences in leaf quality of eight closely related thistles, including those they do not normally encounter under natural conditions. The two populations differed in adult acceptance of, preference for, and larval performance on the various thistle species (Figures 3 and 4, Tables 2 and 3), and were strikingly different in the responses of adult beetles to and larval performance on *C. kagamontanum*, the secondary host in Yuwaku. In the Asiu population, adult beetles clearly avoided *C. kagamontanum* (Figures 2 and 4), and no larva could complete development on it (Table 3). For the Yuwaku population, which depends subsidiarily on *C. kagamontanum*, however, adult acceptance of and preference for *C. kagamontanum* were relatively higher than other potential hosts (Figures 2 and 3), and larval performance on *C. kagamontanum* was low, although a few individuals (c. 10%) were able to emerge as adults (Table 3). This indicates that the Yuwaku population has evolved a feeding preference for and physiological adaptation to *C. kagamontanum*, as it has been utilizing this thistle species as a secondary host under natural conditions.

While the two populations differed in host plant acceptance patterns due to their adaptation to the low-ranked local host (Table 2), they showed similar patterns in the ranking of adult acceptance of (Figure 2) and preference for (Figures 3 and 4), as well as larval performance (Table 3) on potential host plants. It therefore appears that the *E. niponica* populations that depend on different hosts in each locality are 'evolutionarily conservative' (Thompson, 1993) in feeding preference and physiological adaptation to their 'ancestral' host plants. This is similar to the case of the western anise swallowtail butterfly *Papilio zelicaon* (Thompson, 1993). For the Yuwaku and Asiu populations, the adult no-choice (Figure 2) and multiple-choice (Figures 3 and 4) assays showed that the preference ranking was high in the thistle species of subsection Nipponocirsium. In central Honshu, *E. niponica* populations occurred primarily on species of this subsection, except for the Asiu population (M. Yamashita, unpubl.). Although it is unknown whether subsection Nipponocirsium is the ancestral host taxon of *E. niponica* in central Honshu, intrinsic preference for the subsection seems to have been retained even in Asiu, where the population depends exclusively on plants of the subsection Reflexae.

It is also notable that the less-preferred *C. ashiuense* was superior as larval food for both the Asiu and the Yuwaku populations. This indicates that the adult feeding preference for and larval physiological adaptation to *C. ashiuense* evolved independently. A low adult preference for the host plant that is favorable for larval survival is also found in several other species, including nymphaline (Singer, 1971) and *Papilio* butterflies (Wiklund, 1974), and Colorado potato beetle (Hsiao, 1982).

Direct observation of adult feeding behavior indicated that in many cases, palpating frequency were closely equivalent among thistle types, but percentages of biting after palpation were strikingly different (Figures 3 and 4). This suggests that some factor(s) on the leaf surface, encountered during palpation, are important in determining feeding preference. These factors might include leaf volatiles, compounds present in leaf waxes, and physical traits such as leaf texture (Bernays & Chapman, 1994). We have already isolated the feeding deterrent from the *C. kagamontanum* leaves and identified it as pectolinarigenin 7-O-rutinoside (R. Nishida, H. Ono, A. Ito, T. Amano, T. Nishida, Y. Kuwahara, K. Yokokawa, S. Koji and K. Nakamura, unpubl. data).

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