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Differentiation in the ability to utilize *Pterostyrax hispida* (Ebenales: Styracaceae) among four local populations of the phytophagous ladybird beetle *Henosepilachna yasutomii* (Coleoptera: Coccinellidae)

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Abstract The oligophagous ladybird beetle *Henosepilachna yasutomii* Katakura (Coleoptera: Coccinellidae) shows inter-population differences in its host-use. In this beetle, populations using the deciduous tree *Pterostyrax hispida* Sieb. et Zucc. (Styracaceae) were recently rediscovered in the Kanto districts of central Honshu, Japan. In the present study, the ability to utilize *P. hispida* and other host plants was compared among four populations of *H. yasutomii* occurring on *P. hispida* and *Scopolia japonica* Maxim. (Solanaceae), *Chelidonium japonicum* Thunb. (Papaveraceae), and *Solanum tuberosum* L. (Solanaceae). As regards the feeding habits of adult beetles, only the populations occurring on *P. hispida* accepted this plant's leaves, which differed distinctly from the feeding habits of the other populations, although the differences among the four populations were not readily apparent with respect to the leaf amounts consumed. The larvae from the populations occurring on *P. hispida* showed significantly higher survivorship on this plant than did the larvae from the other populations. Considering the host

use patterns and the life cycles of beetles under natural conditions, the large abundance of *P. hispida* leaves throughout the season may have played an important role in selection for the ability to utilize *P. hispida* observed in the *H. yasutomii* populations occurring on this woody host.

Keywords Adult feeding acceptance · Adult feeding preference · Host plant selection · Insect–plant relationships · Larval performance

Introduction

Many species of phytophagous insects show inter-population differences in their host-use patterns. Most cases appear to reflect the occurrence of particular plants in different localities, yet some populations fail to utilize plants that are nonetheless utilized by others (Fox and Morrow 1981; Jaenike 1990; Bernays and Chapman 1994). Because insect populations occurring on different host plants are likely to be subjected to different selection regimes, improvements would be expected in the ability of insects to utilize their current host plants. Moreover, some alternation would be expected in the ability to use other hosts, due to pleiotropic effects of the genes involved in current host plant utilization (Jaenike 1990; Via 1990; Joshi and Thompson 1995). The degree of diversification among insect populations would depend on the magnitude of gene flow between populations, the degree to which the direction and magnitude of corresponding selective forces differ between populations, and the amount of time that has elapsed since a different type of host was utilized.

Differentiation among populations of phytophagous insects may occur as a consequence of utilizing different hosts, even without geographical isolation. As a

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well-known example, two races of the tephritid fly *Rhagoletis pomonella* (Walsh), which occur on apples and hawthorns, showed some degree of differentiation in terms of adult host preference and larval performance on their respective hosts (Bush 1969, and following works). This case has been considered to be one of a small number of empirical examples of incipient sympatric speciation (Tauber and Tauber 1989; Bush and Smith 1997; Howard and Berlocher 1998). Therefore, the first steps toward understanding how inter-population differentiation and/or speciation occur in phytophagous insects are to investigate differences in host-use patterns and to compare the abilities to utilize different host plants among insects.

The phytophagous ladybird beetle *Henosepilachna yasutomii* Katakura (Coleoptera: Coccinellidae; taxonomic affiliation following Jadwyszczak and Węgrzynowicz 2003) exhibits oligophagous food habits, usually depending on blue cohosh *Caulophyllum robustum* Maxim. (Berberidaceae). The occurrence of *H. yasutomii* populations on plants other than *C. robustum* as both main and subsidiary hosts has been frequently reported (for reviews see: Katakura 1981, 1997; Hoshikawa 1983; Tomioka 1986a, 1986b). These plants are taxonomically inconsistent, varying from Papaveraceae (*Chelidonium japonicum* Thunb.) to Cucurbitaceae (*Schizopepon bryoniaefolius* Maxim.), Araliaceae (*Panax japonicus* C. A. Mey.), and Solanaceae (deadly nightshade *Scopolia japonica* Maxim., Chinese matrimony vine *Lycium chinense* Miller, and *Solanum* spp.). Some populations showing different host-uses have sometimes been regarded as different races or biotypes (Katakura 1981; Tomioka 1986a, 1986b; Matsumoto et al. 2004).

In Kanto, central Honshu, Japan, we recently rediscovered populations of *H. yasutomii* occurring mainly on a deciduous tree, *Pterostyrax hispida* Sieb. et Zucc. (Styracaceae) (Matsumoto et al. 2004); there has been only one prior report on one of these populations, which was published approximately 50 years ago (Inoue 1955). This is a quite unique case of the utilization of woody host plants, not only by *H. yasutomii*, but by other related species distributed throughout Japan that occur basically on herbaceous host plants (Katakura 1981; Hoshikawa 1983). In the present study, we carried out simple feeding tests and reared larvae using *H. yasutomii* individuals from populations occurring on *P. hispida* and *S. japonica*, *C. japonicum*, and potato *Solanum tuberosum* L., in the western suburbs of the Tokyo metropolis (Inoue 1952; Yasutomi 1973; Shimbo 1977; Matsumoto et al. 2004) to better understand the ecological and evolutionary processes of differentiation among *H. yasutomii* populations. We paid particular attention to detecting inter-population differences in adult feeding preferences for *P. hispida* and in the ability of larvae to develop on *P. hispida* in order to elucidate how these aspects characterize the populations inhabiting the area concerned.

Materials and methods

Insects and plants

H. yasutomii is a univoltine coccinellid species endemic to Japan (Katakura 1981, 1997; Jadwyszczak and Węgrzynowicz 2003). The general life cycle of this species is roughly the same among populations occurring on different host plants and/or in different localities (Katakura 1981; Shirai 1988; Tamura and Takeuchi 1992; Shirai and Morimoto 1999). Posthibernating adults appear in early spring (mid-April to early May) and initiate oviposition after a short period of voracious feeding. Oviposition continues until mid-summer. Females lay eggs in masses, usually on the undersides of the leaves of their food plants. Adults and larvae feed on the same plant species. New adults start to emerge in early July, and they enter hibernation in autumn, in synchronicity with the withering of the host plant. Virtually every population of *H. yasutomii* can feed and develop normally on *C. robustum* leaves, regardless of the actual host plant(s) being utilized under natural conditions (Yasutomi 1973; Katakura 1981; Hoshikawa 1983; Tomioka 1986a, 1986b). Therefore, in the present study, we treated *C. robustum* as a common host plant for *H. yasutomii*.

In the spring of 2002, posthibernating adult beetles of *H. yasutomii* were collected at four locations in the western suburbs of the Tokyo metropolis (Fig. 1). At each location, the plants listed below were the only confirmed hosts or potential hosts of *H. yasutomii*.

1. Hikawa (Okutama Town): *P. hispida* and *S. japonica* were abundant and sometimes grew sympatrically at high density. Many beetles feeding on both plants were observed. At this location, the relative abundance of leaves was probably higher in the case of woody *P. hispida* than in the case of herbaceous *S. japonica*, and thus the density of beetles per leaf abundance appeared to be lower on *P. hispida* than *S. japonica*. Beetles were collected on *P. hispida* (13 females and 15 males) and on *S. japonica* (12 females and 11 males).
2. Nippara (Okutama Town): *P. hispida* and *S. japonica* were observed, but they occurred somewhat sporadically, i.e., at a lower density than at the former location. These two plants were at least 10 m or more apart from each other. Beetles were observed feeding on both plants, although their density was lower on these plants than at the former location (Hikawa), particularly on *P. hispida*. Therefore, the beetles were collected primarily on *S. japonica* (12 females and 13 males), but one was also obtained on *P. hispida* (1 female). *P. japonicus* was also observed sporadically, but no epilachnine beetles were confirmed on this plant species.

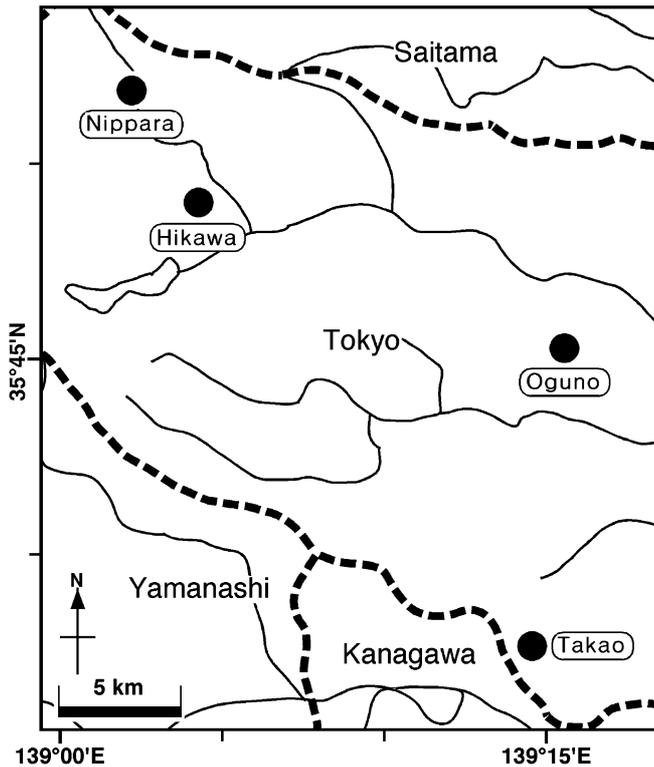


Fig. 1 Localities of the four *Henosepilachna yasutomii* populations (black symbol) investigated in the present study

3. Oguno (Hinode Town): Adults of *H. yasutomii* were collected exclusively on *S. tuberosum* at two small crop fields within a radius of ca. 1 km (22 females and 7 males).
4. Takao (Hachioji City): Adults of *H. yasutomii* were collected exclusively on *C. japonicum*. In the case of this population, the number of beetles collected was rather small (4 females and 5 males), due to their extremely low population density under natural conditions. It has been reported that beetles inhabiting this location also occur on *L. chinense* and *S. tuberosum*, which grow naturally and are cultivated in that region (Tamura and Takeuchi 1992).

As regards the beetles collected at Hikawa and Nippara, our preliminary analyses detected no differences between individuals collected on *P. hispida* and those collected on *S. japonica*, with respect to the acceptability of adults for several host plants and the ability of their offspring to develop on *P. hispida* and *C. robustum* (see below). Therefore, the beetles collected at each location were treated as a single population, regardless of the host plants on which they were collected. As regards the Oguno population, a significant proportion of collected beetles were found to have dipteran parasitoids (species unidentified) and unfortunately died during the experimental period. The high parasitism rate in this population left us with only a small sample for the feeding tests (see below); the data obtained from parasitized individuals, if those existed, were excluded from the analyses when necessary.

Experiments

All of the experiments were carried out under a controlled regime of a L16:D8 cycle at 20°C.

Feeding test 1: non-choice design

Non-choice feeding tests were carried out to assess the differences in plant acceptability among beetle populations. In this experiment, we examined *C. japonicum* (the host plant of the Takao population), *P. hispida* and *S. japonica* (of the Hikawa and Nippara populations), and the common host plant *C. robustum*. Furthermore, as potential hosts that are known to be utilized by other local populations of *H. yasutomii*, we also examined *P. japonicus* (Katakura 1981; Hoshikawa 1983; Tomioka 1986a, 1986b) and the thistle *Cirsium kamtschaticum* Ledeb. (subsidiary utilization of *Cirsium* sp. by populations in northern Honshu, the main island of Japan, has been observed; Y. Tomioka and K. Yasutomi, personal communication). All of the plant materials were collected in and around Sapporo, Hokkaido, northern Japan. For the plant species that are not distributed in Hokkaido (i.e., *C. japonicum*, *P. hispida*, and *S. japonica*), we used leaves from individuals that had been transplanted 15 or more years ago in an experimental garden on the campus of Hokkaido University.

A piece of leaf (about 20 cm²) was placed in a transparent polystyrene case (8.0×15.5×3.0 cm), the bottom of which was covered with moist filter paper. A beetle was released into the case and was allowed to feed on the leaf for 24 h. Each beetle was tested twice for each plant species on 2 successive days. Prior to each 2-day test, the beetle was fed on an alternative food plant, *Solanum japonense* Nakai (Katakura and Hosogai 1997; Fujiyama and Katakura 2002), for more than 24 h, in order to minimize the possible influence of preceding feeding experience and to confirm that the beetle retained normal feeding activity; data obtained from individuals that did not feed on this plant before each experiment were excluded from the analyses.

The leaf area consumed was measured with the aid of image processing software (NIH Image ver. 1.62; National Institutes of Health, Bethesda, Md., USA), after the leaves were photocopied and were scanned into a computer. Differences in thickness or water content between plant species create differential biases in terms of feeding areas. To minimize such biases, the areas measured were converted to wet weights (mg) by multiplying plant species-specific coefficients obtained in our preliminary study.

For each population and each plant species, all collected beetles were investigated simultaneously. Within each population, the order of tests examining different plant species was haphazard.

Type I ANOVAs were performed after log (amounts consumed + 1) transformation, in order to detect the differences in acceptability for the six plant species

within each population, and to detect differences in the patterns of acceptability among populations as an interaction term of population \times plant species.

Feeding test 2: choice design

Choice feeding tests were carried out in order to assess the differences among beetle populations in terms of their preference for *P. hispida*. The methods used were basically those described for feeding test 1. In this series of experiments, a piece of *P. hispida* leaf and a piece of *C. robustum* leaf were offered simultaneously to each beetle. Then, the preference for *P. hispida* relative to the preference for *C. robustum* was determined for each population; the existence of a preference for either plant, as determined by a relatively larger amount consumed, was analyzed by *t*-test for paired comparisons.

Larval performance

In order to assess differences among *H. yasutomii* populations in terms of their ability to develop on *P. hispida*, we reared larvae on the leaves of *P. hispida* and *C. robustum*. Two sets of newly hatched larvae, each containing five individuals from a single egg mass, were prepared and reared on the leaves of either plant species, with one set of larvae per species. Each set of five larvae was reared in a case of the same size and humidity as that used in the feeding tests. We examined each single egg mass from each female beetle investigated in the feeding tests. Therefore, the number of replications varied among populations and ranged from 4 to 11, according to the number of collected (and oviposited) females.

Larvae were reared until the first molting. The number of individuals that reached the second larval stage was recorded for each population and food plant.

Differences in the frequencies of individuals that reached the second larval stage and those that did not between the two food conditions were analyzed by Fisher's exact test for each population, and by log-linear models (Sokal and Rohlf 1995) among populations, in order to examine the interaction term of population \times food plant.

Results

Feeding test 1

For all four populations investigated, the beetles accepted *S. japonica* most, followed by *C. robustum* and *C. japonicum*, whereas acceptance for *P. hispida*, *P. japonicus*, and *C. kamtschaticum* was low (Fig. 2). The pattern of acceptability for the six plant species did not significantly differ among the populations (Table 1; population \times offered plant interaction term was not significant; $P > 0.05$). The acceptability for *P. hispida*

was low, even in the Hikawa and Nippara populations (Fig. 2), despite the fact that this plant species is used by these populations under natural conditions. For these two populations, more than half of the examined beetles did not feed at all on the leaves of this plant species (Table 2). As regards the Oguno and Takao populations, no individual accepted *P. hispida* among the restricted number of beetles examined (Fig. 2; Table 2).

Feeding test 2

The beetles from all four populations strongly preferred *C. robustum* to *P. hispida* (Fig. 3). Nevertheless, no individuals in the Oguno and Takao populations fed on *P. hispida* at all, while some individuals from the Hikawa and Nippara populations fed on both *C. robustum* and *P. hispida*, or only on *P. hispida* (Fig. 3).

Larval performance

For all four populations, there was a significant difference in terms of survivorship to the second larval stage between the two rearing conditions ($P < 0.001$ by Fisher's exact test for each population); larval performance was better on *C. robustum* than on *P. hispida* (Table 3).

In addition, analysis by log-linear models detected significant differences among the populations in the survivorship on the two food plants ($df = 3$, $G = 16.6$, $P = 0.001$). When the larvae were reared on *P. hispida*, more than 60% of the individuals reached the second larval stage in the Hikawa and Nippara populations; on the other hand, in the Oguno population, almost all of the larvae died before the first molting, and none of the individuals in the Takao population survived the first larval stage (Table 3). The rate of survival to the second larval stage on *C. robustum* was high for all of the four populations investigated (more than 90%; Table 3).

Discussion

The four populations of *H. yasutomii* investigated in the present study occur on different host plants or sets of host plants under natural conditions. Therefore, some differentiation was expected among these populations in terms of their ability to utilize actual and potential host plants, because each population is likely to have been subjected to a different selection regime, according to differences in actual host-use. The changes in the ability to utilize potential hosts could have occurred due to pleiotropic effects of genes involved in current host-use, and the ability to utilize certain hosts could have improved in the case of the current host plants (Jaenike 1990; Via 1990; Joshi and Thompson 1995).

When the binary response of adult beetles to *P. hispida* (i.e., whether the beetles accepted this plant or not, regardless of the amount consumed) was

Fig. 2 Mean leaf amounts of six plant species consumed on 2 successive days by posthibernating adult beetles of *Henosepilachna yasutomii* in non-choice feeding tests. Vertical lines added to each bar represent +SE. Sample size (*n*) is given in parentheses. Cr *Caulophyllum robustum*, Cj *Chelidonium japonicum*, Ph *Pterostyrax hispida*, Pj *Panax japonicus*, Sj *Scopolia japonica*, and Ck *Cirsium kantschaticum*. For the Hikawa and Nippara populations, bars with the same letters are not significantly different ($P > 0.05$) by Scheffé's method after $\log(x + 1)$ transformation (data for the plant species with a mean value of zero were excluded from analyses). For the Oguno and Takao populations, statistical analyses were not conducted due to insufficient sample sizes

considered, it appeared that the Hikawa and Nippara populations were slightly but clearly different from the other two populations (Figs. 2, 3, Table 2). Furthermore, striking inter-population differences existed in the ability of larvae to develop on *P. hispida*; larvae from the Hikawa and Nippara populations showed higher survivorship rates on *P. hispida* than did the larvae from the other populations, although the proportion of individuals reaching the second larval stage was significantly smaller on *P. hispida* than on *C. robustum* for both of these populations, as well as for the Oguno and Takao populations (Table 3). Therefore, populations occurring on *P. hispida* in the region of interest could be clearly characterized by their ability to utilize this plant.

On the other hand, considering the amount consumed by adult beetles, no obvious differences among populations were detected in the non-choice feeding test (Fig. 2, Table 1) or in the choice feeding test (Fig. 3). This finding could be primarily due to the responses of the beetles from the populations occurring on *P. hispida* to plants other than *P. hispida*; in other words, a strong similarity in general feeding patterns among populations was observed in terms of the relative acceptability for the six plants (Fig. 2). Moreover, an extremely low preference for *P. hispida* was observed, even among the Hikawa and Nippara populations (Fig. 3), a finding which rendered it difficult to detect inter-population differences. Thus, the present results imply that the pleiotropic effects of the genes involved in the ability to utilize *P. hispida*, if any, are negligible, in particular when these results are considered together with the observation that the ability of the larvae from the Hikawa and Nippara populations to develop on *P. hispida* appears to have

Table 1 ANOVA table for consumed amounts of leaves (mg) by posthibernating adult beetles of *Henosepilachna yasutomii* in non-choice feeding tests. Data were $\log(x + 1)$ transformed before analysis

Factor	df	SS	F
Population	3	2.08	2.23
Plant offered	5	77.60	49.99***
Population×plant offered	15	5.38	1.16
Sex	1	7.26	23.38***
Error	380	117.97	

*** $P < 0.001$

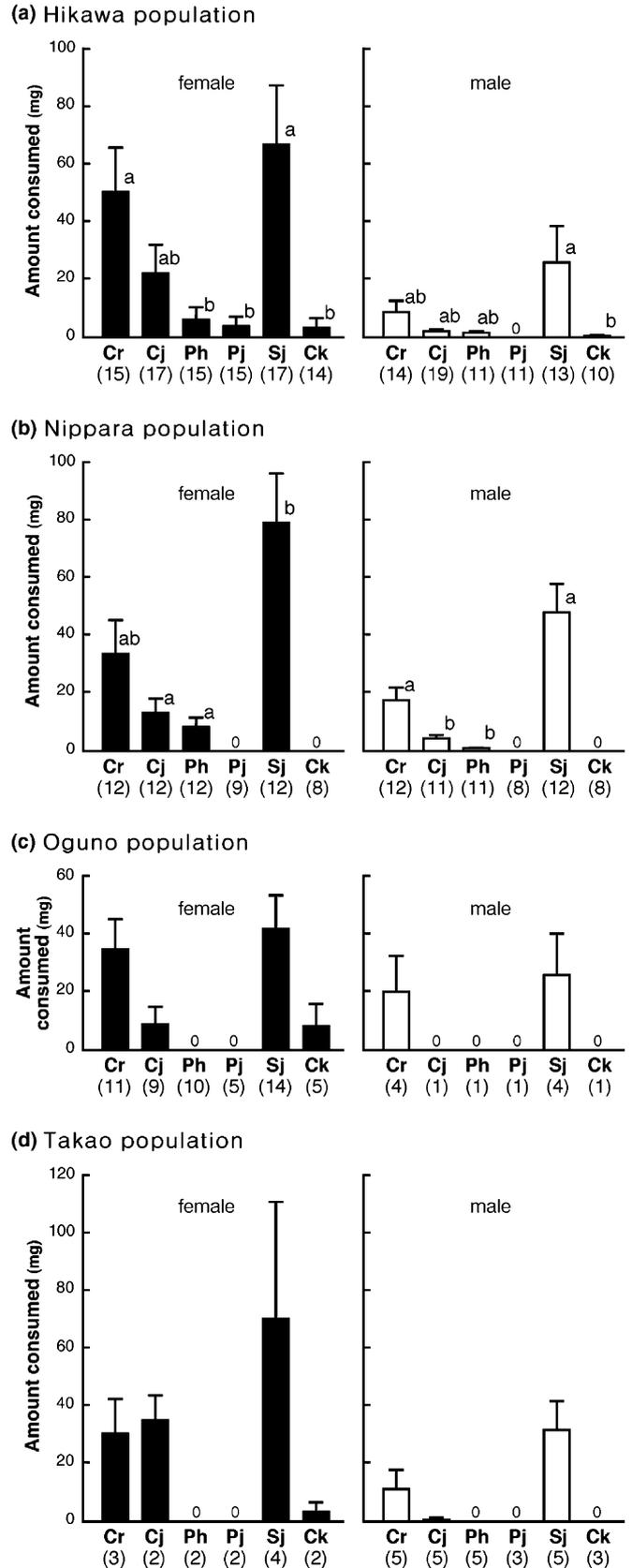


Table 2 Percentage of posthibernating adult beetles of *Henosepilachna yasutomii* that exhibited feeding activity in non-choice feeding tests. The number of beetles investigated (*n*) is given in parentheses

Plant offered	Population							
	Hikawa		Nippara		Oguno		Takao	
	Female	Male	Female	Male	Female	Male	Female	Male
<i>Caulophyllum robustum</i>	66.7 (15)	50.0 (14)	91.7 (12)	91.7 (12)	81.8 (11)	75.0 (4)	100 (3)	40.0 (5)
<i>Chelidonium japonicum</i>	82.4 (17)	42.1 (19)	66.7 (12)	63.6 (11)	44.4 (9)	0 (1)	100 (2)	20.0 (5)
<i>Pterostyrax hispida</i>	26.7 (15)	18.2 (11)	41.7 (12)	36.4 (11)	0 (10)	0 (1)	0 (2)	0 (5)
<i>Panax japonicus</i>	6.7 (15)	0 (11)	0 (9)	0 (8)	0 (5)	0 (1)	0 (2)	0 (3)
<i>Scopolia japonica</i>	82.4 (17)	69.2 (13)	91.7 (12)	100 (12)	92.9 (14)	100 (4)	100 (4)	100 (5)
<i>Cirsium kamschaticum</i>	7.1 (14)	10.0 (10)	0 (8)	0 (8)	20.0 (5)	0 (1)	50.0 (2)	0 (3)

been achieved without any apparent reduction in their respective ability to develop on *C. robustum* (Table 3) (cf. Ueno et al. 1999, 2001, 2003). If such absence of pleiotropy can be generalized for the genes involved in the ability to utilize various host plants, the differences in host-utilization patterns observed in *H. yasutomii* may have been shaped through simple increases in the number of plants that can be utilized as hosts, or through an independent loss of the ability to utilize a particular host.

There is an intriguing aspect in the host-use by *H. yasutomii* populations investigated here. Based on the laboratory experiment, *P. hispida* appears to be a less suitable host, both in terms of adult feeding (Figs. 2, 3,

Table 2) and larval development (Table 3), than the common host of *H. yasutomii*, *C. robustum*. Furthermore, the general inadequacy of *P. hispida* among host plants of *H. yasutomii* can also be inferred from the lower adult feeding acceptance for the leaves of this plant (Fig. 2, Table 2). The question then arises concerning why the Hikawa and Nippara populations have to utilize *P. hispida* under natural conditions. A similar phenomenon has been reported in a related species of *H. yasutomii* in Southeast Asia; populations of *H. vigintioctopunctata* (Fabricius) depending solely on an introduced weed, *Centrosema pubescens* Benth. (Fabaceae), exist under natural conditions, despite the fact that the suitability of *C. pubescens* determined in the laboratory

Fig. 3 Choices made by posthibernating adult beetles of *Henosepilachna yasutomii* for *Pterostyrax hispida* and *Caulophyllum robustum*. Each dot represents leaf amounts consumed by each beetle on 2 successive days. Note the different scales for *P. hispida* and *C. robustum*. For the Hikawa and Nippara populations, statistically significant differences ($P < 0.05$) indicate the existence of a preference for either plant, as determined by larger amounts consumed (analyzed by *t*-test for paired comparisons). For the Oguno and Takao populations, statistical analyses were not conducted due to insufficient sample sizes

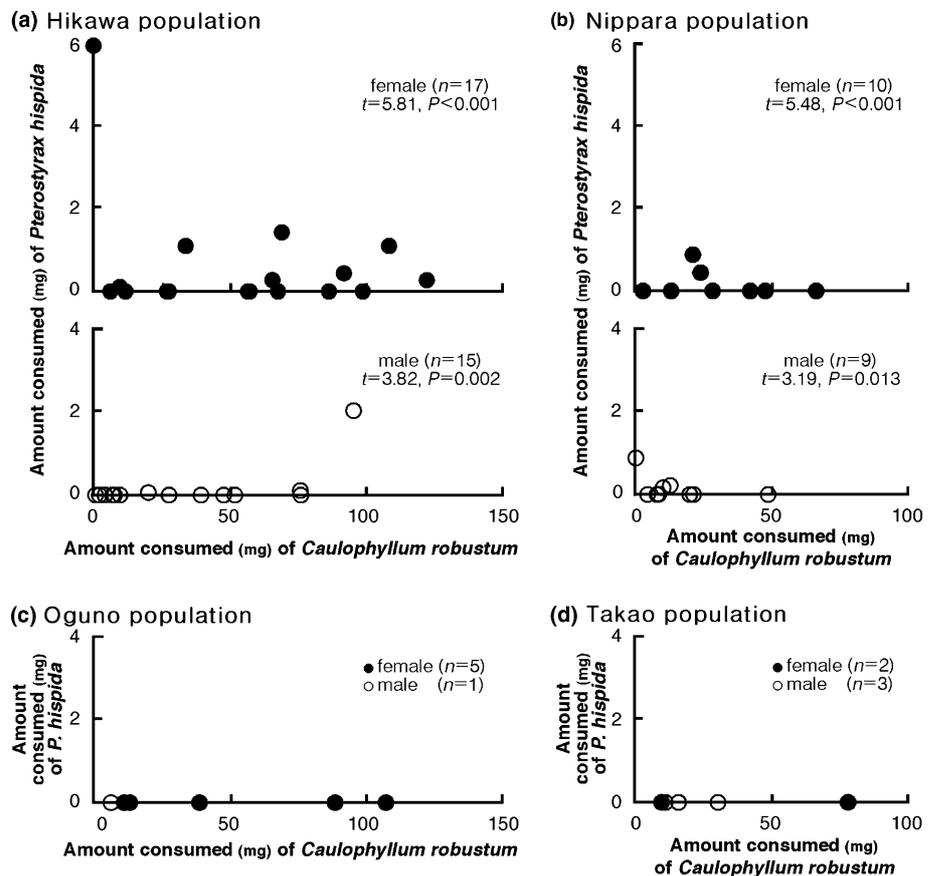


Table 3 Rates of survival (%) to the second larval stage of *Henosepilachna yasutomii* reared on *Caulophyllum robustum* or *Pterostyrax hispida* leaves. The number of individuals examined (*n*) is given in parentheses

Population	Reared on	
	<i>C. robustum</i>	<i>P. hispida</i>
Hikawa	94.6 (55)	63.6 (55)
Nippara	100 (50)	70.0 (50)
Oguno	90.0 (50)	4.0 (50)
Takao	100 (20)	0 (20)

was apparently lower than that of the ordinary solanaceous hosts, e.g., *Solanum torvum* Swartz. (Shirai and Katakura 1999, 2000). For this case, Shirai and Katakura (2000) suggested that certain ecological conditions, such as the abundance and availability of the host plant, or a possible scarcity of natural enemies on the host plant, may compensate for the lower quality of the host plant. The relevant ecological characteristics of *P. hispida* have not yet been precisely investigated. However, at least due to its woodiness, this host plant appears to provide a more abundant food resource for beetles until later in the season than do the other herbaceous host plants of *H. yasutomii*, e.g., *C. robustum*, *S. japonica*, or cultivated potato *S. tuberosum*, of which availability and abundance are limited to a somewhat earlier season. Thus, the huge abundance of *P. hispida* leaves may have promoted the utilization of this plant by some populations of *H. yasutomii*, despite the possible inadequacy of leaf quality.

Even though the Hikawa and Nippara populations appeared to be able to utilize *P. hispida* without any physiological changes in their ability to use other host plants, there may exist another type of adaptation to *P. hispida* with respect to life history traits. *H. yasutomii* depends generally on herbaceous plants, the availability and abundance of which are limited to an earlier season, as just mentioned, and the life cycle of *H. yasutomii* populations appears to be well adapted to the phenology of the host plants (Katakura 1981; Shirai 1988; Tamura and Takeuchi 1992; Shirai and Morimoto 1999). However, as regards populations occurring on *P. hispida*, an extension of activity period within their univoltine life cycle (i.e., the longer active season for both posthibernating and newly emerged adults, and the longer period for larval development) has been suggested by our field observations (Matsumoto et al. 2004). Furthermore, at locations such as Hikawa, where beetles occur on both *P. hispida* and *S. japonica* in sympatry, virtually all beetles appeared to eventually utilize *P. hispida* because of the exhausting and/or withering of *S. japonica* by July at the latest, and gene flow between beetles feeding on the different hosts can occur (K. Matsumoto, unpublished data). There remains the possibility that the elongation of activity period on *P. hispida* may merely be the result of the inadequacy of the leaf quality of this plant. However, if the *H. yasutomii* populations occurring on *P. hispida* have once evolved a life cycle that

enables them to utilize this woody host plant until late in the season, they will consequently fail to depend solely on other herbaceous host plants that are available only earlier in the season. Therefore, the populations occurring on *P. hispida* may have been differentiated from the other populations occurring on herbaceous plants, not only in the physiological ability to utilize *P. hispida* observed in the present study, but also in other ecological traits. Further studies focusing in particular on the demographic characteristics of each population will be needed to elucidate the ecological differences among the four populations of *H. yasutomii* examined here and to estimate the process of diversification in host plant utilization patterns observed in *H. yasutomii*.

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