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Host Preference of F_1 Hybrids between Two Host Specific Phytophagous Ladybird Beetles (*Epilachna* spp., Epilachninae, Coccinellidae)

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Abstract We reared *Epilachna niponica*, *E. yasutomii* and their F_1 hybrids throughout the larval stage on thistle (*Cirsium kamtschaticum*, the host plant of *E. niponica*), blue cohosh (*Caulophyllum robustum*, the host plant of *E. yasutomii*) or Japanese nightshade (*Solanum japonense*, a plant preferred by both species in the laboratory). When allowed to choose between the three plants, the majority (80–100%) of *E. niponica* and *E. yasutomii* fed on their own host plants and the Japanese nightshade, but none accepted the host plants of the other species. About one third of the individuals of either species accepted the host plants of the other species when no choice was offered. On the other hand, hybrids mostly preferred nightshade, followed by blue cohosh. Although they least preferred thistle, a considerable proportion (ca. 15–65%) ate this plant even when they were allowed to select between all three. When given no choice, all but one individual of the hybrids accepted all the three plants. The effect of pre-imaginal conditioning on adult host preference was weak, if it existed at all. However, the possibility of hybridization in natural conditions and its effect on the reproductive isolation between *E. niponica* and *E. yasutomii* remains unclear.

Key words: *Epilachna niponica*; *Epilachna yasutomii*; F_1 hybrids; preference; host plants

Introduction

Reproductive isolation in phytophagous insects can be totally dependent on difference in host plants. This is true for a pair of sympatric Japanese phytophagous ladybird beetles *Epilachna niponica* LEWIS and *E. yasutomii* (KATAKURA). In natural conditions, *E. niponica* depends on thistles (*Cirsium* spp., Compositae) as a food source, whereas *E. yasutomii* feeds on blue cohosh (*Caulophyllum robustum* MAXIM., Berberidaceae) and other local subsidiary host plants such as *Chelidonium japonicum* THUNB. (Papaveraceae), *Panax japonicus* C. A. MEYER (Araliaceae) and *Scopolia japonica* MAXIM. (Solanaceae) (KATAKURA, 1981; HOSHIKAWA, 1983). Under laboratory conditions, adults preferred and larvae survived much better on their respective host plants (KATAKURA *et al.*, 1989). Furthermore, both species usually mate on their host plants. No evidence for sexual or gametic isolation or reduced hybrid

fertility was detected (KATAKURA *et al.*, 1981, 1989). KATAKURA *et al.* (1989) concluded that the two species are reproductively isolated by the host difference alone. To supplement the results obtained by KATAKURA *et al.* (1989) and KATAKURA & HOSOGAI (1994), we analyzed the preference of the F_1 hybrids. In the present paper, we show that the host preference of F_1 adults expands considerably to cover the host plants of both parental species.

Materials and Methods

Insects. We tested the host preference of a total of 29 individuals of *E. niponica*, 36 individuals of *E. yasutomii* and 81 individuals of F_1 hybrids obtained by two reciprocal crossings. Each species or type of hybrid consisted of three groups of beetles reared on different plants throughout their larval stages. The first and second groups were reared on fresh picked leaves of thistle (*Cirsium kamtschaticum* Ledeb.) and blue cohosh, respectively, under 16L8D at 20°C. The larval survival rate was reported by KATAKURA & HOSOGAI (1994). The third group was reared in a greenhouse on potted Japanese nightshade (*Solanum japonense* NAKAI, Solanaceae), an alternative food plant preferred by both species under laboratory conditions.

All tested beetles were offspring of *E. niponica* and *E. yasutomii* collected respectively on thistle (*Cirsium inundatum alpicola* (NAKAI) KITAM.) and blue cohosh at Ohnuma, Oshima Peninsula, southernmost Hokkaido, Japan (see, KATAKURA & HOSOGAI, 1994).

Methods. We examined the preference for thistle, blue cohosh and Japanese nightshade of newly emerged adults as follows: Since adults do not take food until about two days after emergence, beetles two days after adult eclosion were individually kept in a styrene cage and allowed to choose between fresh leaves of thistle, blue cohosh and Japanese nightshade for three days (Period I). Leaves were changed daily, and daily leaf consumption (feeding intensity) by each individual was scored as follows (KATAKURA *et al.*, 1989): no feeding = 0; consumed leaf area up to 0.25 cm^2 = 1; up to 1.0 cm^2 = 2; up to 4.0 cm^2 = 3; and more than 4.0 cm^2 = 4. When a beetle did not consume one or two plants at all, or ate only once and with an intensity of 1 (namely, it consumed less than 0.25 cm^2 during Period I), the experiment was continued to ascertain whether the beetle would not accept the plant(s) even if the preferred plant(s) was not available (Period II). When a beetle did not consume one plant during Period I, the beetle was subsequently offered the plant for up to three days (no-choice test). The beetle was treated as not accepting the plant if it did not consume the plant for the three successive days. When a beetle did not comsume two plants, the beetle was allowed to choose between the unaccepted plants for up to three days. When one of the two plants was left uneaten during the choice test, the

Table 1. Food preference of newly emerged adults of the ladybird beetles *Epilachna niponica*, *E. yasutomii* and their F_1 hybrids reared on three species of plant, blue cohosh (BC, host plant of *E. yasutomii*), thistles (TH, host plant of *E. niponica*) and Japanese nightshade (NS). Each beetle was allowed to choose between the three plants during the first three days (Period I), and then forced to accept plant(s) that was not eaten during Period I for up to six days (Period II). Feeding intensity (per individual per day) is for individuals that had accepted the plant concerned. N=The number of individuals examined. NY hybrids: F_1 hybrids between *E. niponica* females and *E. yasutomii* males; YN hybrids: those between *E. yasutomii* females and *E. niponica* males. Further explanations in text.

Beetle species	Reared on	Percentage of individuals accepting and feeding intensity (in parentheses) when given choices between the three plants (Period I)				Percentage of individuals accepting throughout experiment (Period I+II)		
		N	TH	BC	NS	TH	BC	NS
E. niponica	TH	16	87.5 (2.0)	0 —	100.0 (2.5)	100.0	25.0	100.0
	BC	2	100.0 (2.5)	0 —	100.0 (1.8)	100.0	50.0	100.0
	NS	11	81.8 (2.1)	0 —	100.0 (2.7)	100.0	36.4	100.0
	Total	**29**	**86.2**	**0**	**100.0**	**100.0**	**31.0**	**100.0**
NY hybrids	TH	11	63.6 (0.8)	90.0 (1.8)	100.0 (2.2)	100.0	100.0	100.0
	BC	7	14.3 (0.7)	100.0 (2.1)	100.0 (2.0)	100.0	100.0	100.0
	NS	12	16.7 (0.3)	100.0 (1.7)	100.0 (2.6)	100.0	100.0	100.0
	Total	**30**	**33.3**	**96.7**	**100.0**	**100.0**	**100.0**	**100.0**
YN hybrids	TH	10	60.0 (1.6)	70.0 (1.6)	90.0 (2.9)	100.0	90.0	100.0
	BC	12	33.3 (0.8)	91.7 (2.7)	83.3 (1.8)	100.0	100.0	100.0
	NS	29	55.2 (0.9)	93.1 (1.7)	100.0 (2.4)	100.0	100.0	100.0
	Total	**51**	**51.0**	**88.2**	**94.1**	**100.0**	**98.0**	**100.0**
E. yasutomii	TH	2	0 —	100.0 (2.2)	100.0 (2.0)	100.0	100.0	100.0
	BC	11	0 —	90.9 (2.1)	100.0 (1.9)	36.4	100.0	100.0
	NS	23	0 —	100.0 (2.8)	78.0 (1.7)	26.1	100.0	100.0
	Total	**36**	**0**	**97.2**	**91.7**	**33.3**	**100.0**	**100.0**

Table 2. Number of individuals which consumed thistle (TH), thistle and blue cohosh (TH+BC), and blue cohosh (BC) during Period I. Data are from Table 1. Four individuals of *E. niponica* (two reared on thistle and two on nightshade) feeding only on nightshade were excluded. NY hybrids: F_1 hybrids between *E. niponica* females and *E. yasutomii* males; YN hybrids: those between *E. yasutomii* females and *E. niponica* males. N=The number of individuals examined.

Beetle species	Number of individuals accepting			
	N	TH	TH+BC	BC
E. niponica	25	25	0	0[a]
NY hybrids	30	1	10	19[b]
YN hybrids	51	4	22	25[b]
E. yasutomii	36	0	0	36[c]

Differences between rows with different letters at the right margin are statistically significant (*Chi*-square test, d.f.=2, $P<0.05$).

no choice test was subsequently performed. Period II thus lasted for up to six days in a few beetles.

Results and Discussion

The results are summarized in Table 1. The preferences to thistle and blue cohosh are given in Table 2. Results of males and females are pooled.

Epilachna niponica and *E. yasutomii* were distinct in food preference strongly selecting their respective host plants (*E. niponica* = thistle, *E. yasutomii* = blue cohosh), but both species also preferred Japanese nightshade (Table 1). When given choices, each species did not eat the host plants of the other species. When the results of no choice tests were incorporated, all the individuals of *E. niponica* and *E. yasutomii* accepted Japanese nightshade and their respective host plants, and the proportion of individuals which ate the host plant of the other species were limited, being ca. 30%. In both species, there was no clear tendency that the adult beetles prefer plants on which they had fed during the larval stage.

On the other hand, F_1 hybrids were considerably different from their parents in that they accepted a wider range of plants. When given choices, the majority of F_1 hybrids preferred Japanese nightshade, and their next choice was blue cohosh. Feeding intensities for these two plants were also similar. They accepted thistle least, but a considerable proportion (15–65%) ate this plant even when they were given the choice between all three food plant species, although the consumed amount of thistle was smaller than that of the other two plants. When the results of no choice tests were incorporated, all but one individual of the hybrids accepted all three plants. No significant difference was detected in host preference between the two types of F_1 hybrids (Table 2). As to the effects of larval host plants on the adult host preference, the results of the hybrids were ambiguous, although feeding intensities tended to be larger on the plants they experienced during their larval stage (Table 1).

KATAKURA *et al.* (1989) assumed that reproductive isolation owing to the host specificity was very strong between *E. niponica* and *E. yasutomii*, because any other effective isolating mechanisms could not be detected between them. The food preference of the F_1 hybrids reported here and elsewhere (KATAKURA & HOSOGAI, 1994) supports this assumption. It is very likely that F_1 hybrids will grow normally and become fertile adults on the host plant of either parent (KATAKURA & HOSOGAI, 1994), and will contribute to gene flow between *E. niponica* and *E. yasutomii* through their expanded host preference (Table 1). A logical consequence of this process would be a fusion of two formerly distinct populations. However, this is apparently not the case. The two species are sympatric and keep their morphological and biological distinctness in northern

Japan from southern Hokkaido to central Honshu (KATAKURA, 1981; KATAKURA *et al.*, 1989). One explanation for this discrepancy is that host selection is sufficiently strict and completely prevents hybridization of the two species under the natural conditions. Another explanation is that hybrids are produced but, because of their equivocal host preferences, they are considerably less efficient than their parents in exploiting their appropriate host plants and so they are selected against.

Thus, although host preference seems to act as a very strong premating isolating mechanism between *E. niponica* and *E. yasutomii*, it is still not clear whether occasional hybridization occurs or not under the natural conditions. In order to understand the real nature of reproductive isolation between the two species, we need to know much more about various aspects of the life histories of the beetles and their host plants, such as demography, phenological relationship with the host plants, and the density, patchiness and distribution pattern of the host plants.

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