Field-cage experiments on host fidelity in a pair of sympatric phytophagous ladybird beetles

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Abstract

Using field cages, we tested the hypothesis that two phytophagous ladybird beetles, *Henosepilachna niponica* (Lewis) and *Henosepilachna yasutomii* Katakura (Coleoptera: Coccinellidae: Epilachninae), are reproductively isolated from each other by their host fidelity alone. We planted host plants of the two species [the thistle *Cirsium alpicola* Nakai (Asteraceae) for *H. niponica* and the blue cohosh, *Caulophyllum robustum* Maxim. (Berberidaceae), for *H. yasutomii*] in 7 × 7 m cages (BT cages), and planted the two host plants and the beetles' common food plant, the Japanese nightshade, *Solanum japonense* Nakai (Solanaceae), in other cages (BJT cages). We then released overwintered reproducing beetles into these cages and recorded their dispersal, the distribution of egg masses, and the incidence of copulations. In BT cages, the beetles moved almost exclusively between their own host plants, and all females laid eggs exclusively on their own host plants. In BJT cages, the beetles moved between their own host plants and the Japanese nightshade, resulting in occasional interspecific mating on the same nightshade plant. Females of both species laid eggs on their own host plants as well as on the Japanese nightshade. These results indicate that host fidelity functions as a strong barrier against gene flow between *H. niponica* and *H. yasutomii*. However, this barrier can easily be broken down if a third plant species bridges the beetle populations.

Introduction

A variety of theoretical studies predict that sympatric speciation may occur under certain conditions (e.g., Via, 2001; Fry, 2003; Gavrilets, 2004), yet empirical studies are few, relative to theoretical studies (Coyne & Orr, 2004). Phytophagous insects are considered prime candidates for sympatric speciation because of intimate and often highly specialized relationships with their host plants (Tauber & Tauber, 1989; Bush, 1994; Via, 2001; Gavrilets, 2004). The assumption is that new species may arise via the sympatric formation of host-specific races that become reproductively isolated (at least to some extent) solely through fidelity to particular host plants. Hence, it is of crucial importance whether host fidelity can function as a strong reproductive barrier between sympatric species feeding on different host plants.

A group of closely related phytophagous ladybird beetles called the *Henosepilachna vigintioctomaculata* species complex (the *Epilachna vigintioctomaculata* complex in our previous papers; for the alteration of the generic name for this species complex, see Jadwiszczak & Węgrzynowicz, 2003) (Coleoptera: Coccinellidae: Epilachninae) has been considered one of the candidates for sympatric speciation via host races (Diehl & Bush, 1984; Bush & Howard, 1986; Tauber & Tauber, 1989; Drès & Mallet, 2002). Reproductive isolation of a sympatric species pair in this species complex, *Henosepilachna niponica* (Lewis) and *Henosepilachna yasutomii* Katakura, has been studied in detail with populations at Ohnuma, Oshima Peninsula, southern Hokkaido, Japan. In this area, *H. yasutomii* occurs on blue cohosh [*Caulophyllum robustum* Maxim. (Berberidaceae)] growing on shaded forest floors, whereas *H. niponica* occurs on thistle [mainly *Cirsium alpicola* Nakai (Asteraceae)] that grows along forest edges and in grasslands around marshes. It was shown that (1) there is no distinct seasonal isolation between *H. yasutomii* and *H. niponica*, (2) there is no positive mate choice, (3) each species chooses its own host when given a choice between the two host

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plants, (4) larvae do not grow well on the host plant of the other species, and (5) F1 hybrids between the two beetle species grow normally to fertile adults on both host plants under laboratory conditions (Katakura et al., 1989; Katakura & Hosogai, 1994, 1997).

Based on these results, Katakura and his coworkers argued that the two species are reproductively isolated from each other by host fidelity alone (Katakura et al., 1989; Katakura & Hosogai, 1994, 1997; Katakura, 1997), although it is still not clearly understood whether the mode of their speciation was sympatric or allopatric. Host-choice experiments conducted in these studies were made in small containers under laboratory conditions, using picked host leaves. As insects often exhibit unusual behavior in captivity and may respond differently to picked and intact leaves, it is unclear how strict the host choice of the beetles is under natural conditions. Furthermore, reproductive isolation solely by host choice may break down in the circumstance where both blue cohosh and thistle grow in close proximity at the forest edge because there are no effective postmating barriers between the two beetle species.

In addition, there could be a situation where hybridization might be more frequent. It is known that members of the *H. vigintioctomaculata* complex can be reared on some solanaceous plants. If such 'common' food plants exist near blue cohosh and thistle that, respectively, harbor *H. yasutomii* and *H. niponica*, the possibility of encounter of these two species of ladybird beetles would be much greater than in the situation where only blue cohosh and thistle occur. Although no case has so far been documented in which a common food plant was found where the two beetle species exist in sympatry, we wanted to know what might happen when such a situation actually does occur.

In the present study, we tested the hypothesis that the two species are reproductively isolated by their host specificity alone, by using field cages in which host plants were planted to mimic conditions that may occasionally be realized under natural conditions. We released overwintered beetles into these cages and recorded their dispersal, the kinds of plants on which oviposition occurred, and the incidence of copulations.

Materials and methods

Insects

Both *H. niponica* and *H. yasutomii* are univoltine and hibernate as adults (Katakura et al., 1989). Overwintered adults appear in spring and reproduce until early summer. New adults emerge in midsummer and enter hibernation by autumn. In mid-May from 2000 to 2002, we collected overwintered beetles of *H. yasutomii* and *H. niponica* on their respective host plants (*H. yasutomii* on blue cohosh, *C. robustum*; *H. niponica* on thistle, *C. alpicola*) at Ohnuma, Oshima Peninsula, in southern Hokkaido, Japan, just before the start of oviposition. The collected beetles were separated by sex and were maintained with their own host plants for a few weeks until the beginning of experiments in the laboratory under a regulated regime of L16:D8 at 23–25 °C.

Plants

Blue cohosh (*C. robustum*) and thistle (*C. alpicola*) used for cage experiments were transplanted from their original habitats to the campus of the Hokkaido University. Blue cohosh plants were transplanted from the suburbs of Sapporo, and thistle plants from Ohnuma. In addition to these two host plants, we chose the Japanese nightshade (*Solanum japonense* Nakai) as a common food plant for *H. yasutomii* and *H. niponica*. This species is known as a suitable food plant for rearing both *H. yasutomii* and *H. niponica* under laboratory conditions (Katakura & Hosogai, 1997; Fujiyama & Katakura, 2002), although there is no report that this species is fed upon by either species of ladybird beetle in the field. We have maintained the Japanese nightshade used for the experiments in a greenhouse at the Hokkaido University for more than 10 years.

Field-cage experiment

On the campus of Hokkaido University, we set up 7 × 7 m field cages of two types, 'blue cohosh–thistle cage' (BT cage) and 'blue cohosh–Japanese nightshade–thistle cage' (BJT cage). In 2000, we planted in a BT cage 10 plants of blue cohosh in half the area and 10 thistle plants in the other half (BTa, Figure 1). In 2002, we added two additional thistle plants and three blue cohosh plants alternately between the blue cohosh area and the thistle area (BTb, Figure 1). In BJT cages, six Japanese nightshade plants were planted linearly between the blue cohosh and the thistle areas (Figure 1). Cages were covered with white nylon-mesh net, and the blue cohosh areas were further shaded with black cheesecloth net to mimic their habitat (forest floor) under natural conditions.

Beetles were marked with marking pen on the elytra prior to the experiment, then released into the field cages (Table 1). In late May 2000, we released five pairs each of *H. yasutomii* and *H. niponica* into a BTa cage (henceforth abbreviated as BT-YN cage) and a BJT cage (BJT-YN) (Table 1). When a marked adult was confirmed dead or not found for a long time (about 10–14 days), another marked adult of the same sex was released. Because we released females of both species simultaneously in the same cages, and because we were not able to discriminate egg masses of the two species, we could not determine the females' oviposition choice in this experiment. We then conducted

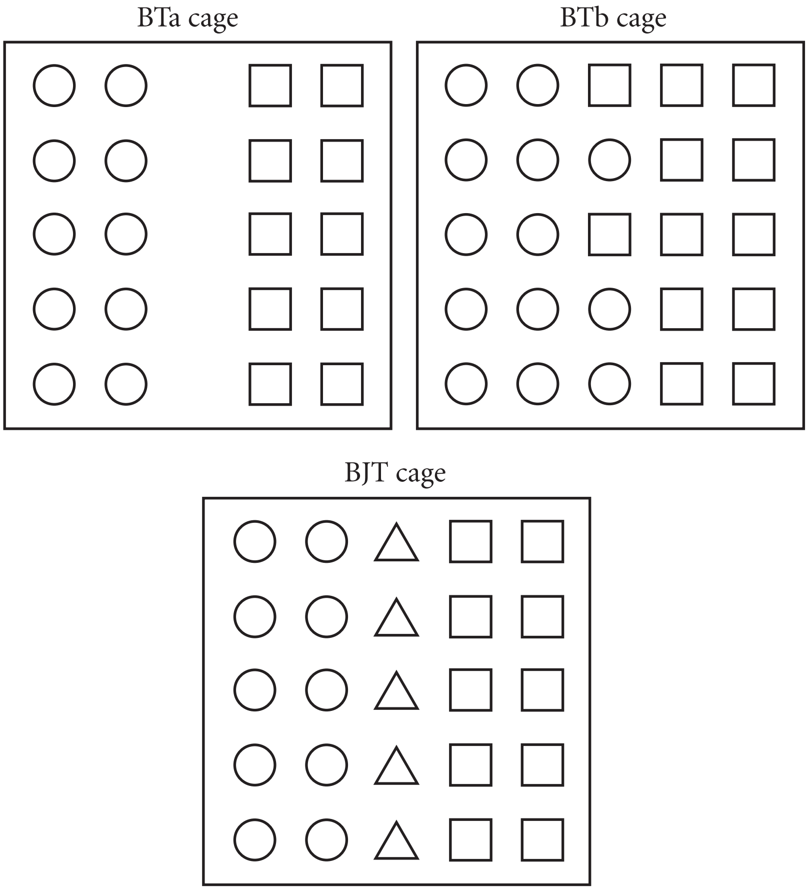

Figure 1 Arrangement of plant species in three cage types (7 × 7 m). Circle, blue cohosh; square, thistle; triangle, Japanese nightshade.

experiments of a different design in 2001 and 2002 (Table 1). In late May 2001, we released 10 females and five males of *H. yasutomii* and five males of *H. niponica* into one BJT cage (BJT-Y), and 10 females and five males of *H. niponica*, and five males of *H. yasutomii* into another BJT cage (BJT-N). In this experiment also, beetles that had died or disappeared were replaced as mentioned previously. Finally, in early June 2002, 20 females of *H. yasutomii* and 10 males each of *H. yasutomii* and *H. niponica* were released into a BTb cage (BT-Y), and 20 females of *H. niponica* and 10 males each of *H. yasutomii* and *H. niponica* into another BTb cage (BT-N). In this experiment, we did not replace beetles that had died or disappeared. Thus, in the experiments conducted in 2001 and 2002, only females of either species were released together with males of both species, permitting precise estimation of females' choice of host plants for oviposition.

Table 1 Experimental periods and conditions of experimental cages

			Number of beetles initially released[a]			
			H. yasutomii		*H. niponica*	
Cage	Cage type	Period of experiment	Female	Male	Female	Male
BT-YN	BTa	31 May–28 September, 2000	5 (7)	5 (9)	5 (5)	5 (6)
BJT-YN	BJT		5 (7)	5 (9)	5 (5)	5 (7)
BJT-Y	BJT	31 May–10 September, 2001	10 (10)	5 (11)	–	5 (5)
BJT-N	BJT		–	5 (11)	10 (12)	5 (7)
BT-Y	BTb	5 June–8 August, 2002	20 (20)	10 (10)	–	10 (10)
BT-N	BTb		–	10 (10)	20 (20)	10 (10)

[a] The total numbers of beetles released during the experiments are given in parentheses.

Table 2 Cumulative numbers of sightings of overwintered adults of *Henosepilachna yasutomii* and *Henosepilachna niponica* on each of the two or three plant species in the field cages

			Plant			
Species	Cage	Sex	Blue cohosh	Japanese nightshade	Thistle	Total
H. yasutomii	BT-YN	Female	250	–	5	255
	BT-YN	Male	172	–	3	175
	BT-Y	Female	337	–	5	342
	BT-Y	Male	190	–	4	194
	BT-N	Male	172	–	17	189
H. niponica	BT-YN	Female	0	–	311	311
	BT-YN	Male	0	–	231	231
	BT-Y	Male	1	–	74	75
	BT-N	Female	0	–	484	484
	BT-N	Male	1	–	203	204
H. yasutomii	BJT-YN	Female	241	59	0	300
	BJT-YN	Male	137	83	3	223
	BJT-Y	Female	228	77	1	306
	BJT-Y	Male	96	10	0	106
	BJT-N	Male	47	43	0	90
H. niponica	BJT-YN	Female	0	174	201	375
	BJT-YN	Male	1	9	183	193
	BJT-Y	Male	1	101	87	189
	BJT-N	Female	0	128	151	279
	BJT-N	Male	0	9	82	91

Every day, we recorded the plants on which beetles or egg masses were found, and the position of beetles. We continued these daily observations until late September in 2000, early September in 2001, and in early August in 2002 (Table 1).

Results

Distribution of overwintered adults

Table 2 shows the distribution of overwintered adults of *H. yasutomii* and *H. niponica* in the field cages, expressed by the cumulative number of sightings counted on each plant during daily observations. Patterns of beetle movements in the BT-YN and BJT-YN cages are illustrated in Figures 2 and 3. Essentially, the same patterns were obtained for the BT-Y, BT-N, BJT-Y, and BJT-N cages (data not shown). Almost all beetles were found on their respective host plants (in BT cages), or on their respective host plants and the common food plant, Japanese nightshade (in BJT cages). Very few individuals were found on the host plants of the other species. Those individuals that were found on the host plants of the other species were observed for up to a few continuous days on the same plant without any trace of feeding (Figures 2 and 3). During the experiment, matings were occasionally found on the respective host plants and the common food plants, but never observed on the host plants of the other species (Table 3).

Table 3 Number of matings observed in the field cages. All conspecific matings were on either blue cohosh (in cases of *Henosepilachna yasutomii*) or thistle (in cases of *Henosepilachna niponica*), whereas all interspecific matings were on Japanese nightshade

	Conspecific mating		Interspecific mating	
Cage	*H. yasutomii*	*H. niponica*	*H. yasutomii* female × *H. niponica* male	*H. niponica* female × *H. yasutomii* male
BT-YN	5	8	0	0
BT-Y	5	–	0	–
BT-N	–	6	–	0
BJT-YN	4	6	0	3
BJT-Y	4	–	2	–
BJT-N	–	3	–	2

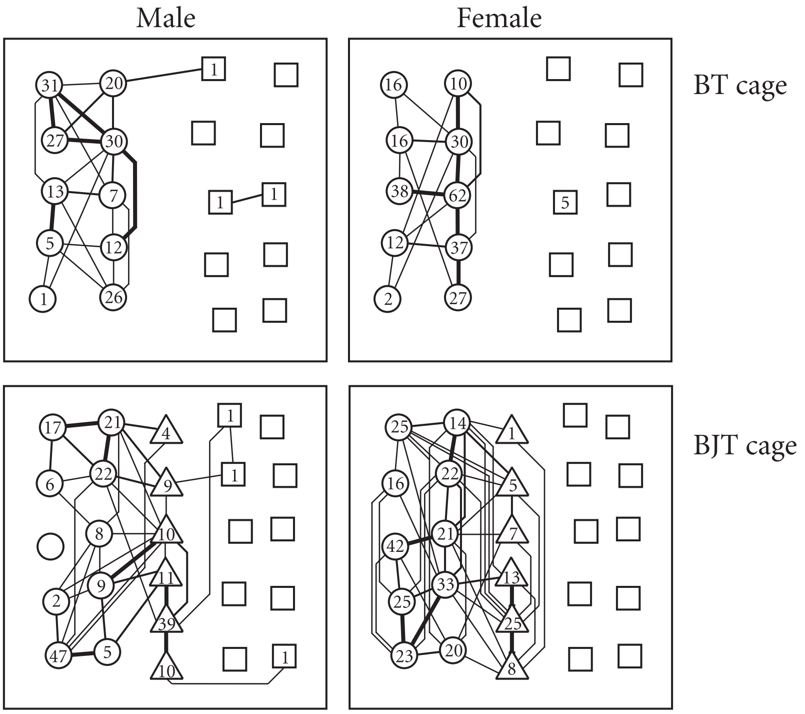

Figure 2 Distribution of adult beetles of *Henosepilachna yasutomii* in the field cages studied in 2000. Numerals indicate cumulative number of sightings of beetles on each plant. Where a beetle was successively observed on two different plants, the two plants are connected by a line. The thickness of line is approximately proportional to the frequency of beetle movement between the two plants.

Distribution of eggs

Henosepilachna beetles lay eggs in masses on the underside of leaves of host plants (Nakano et al., 2001). Table 4 shows the cumulative number of egg masses found on each plant species studied in 2001 and 2002. Eggs were exclusively found on respective host plants (in BT cages) or on respective host plants and the common food plant (in BJT cages). No egg masses were found on the host plant of the other species.

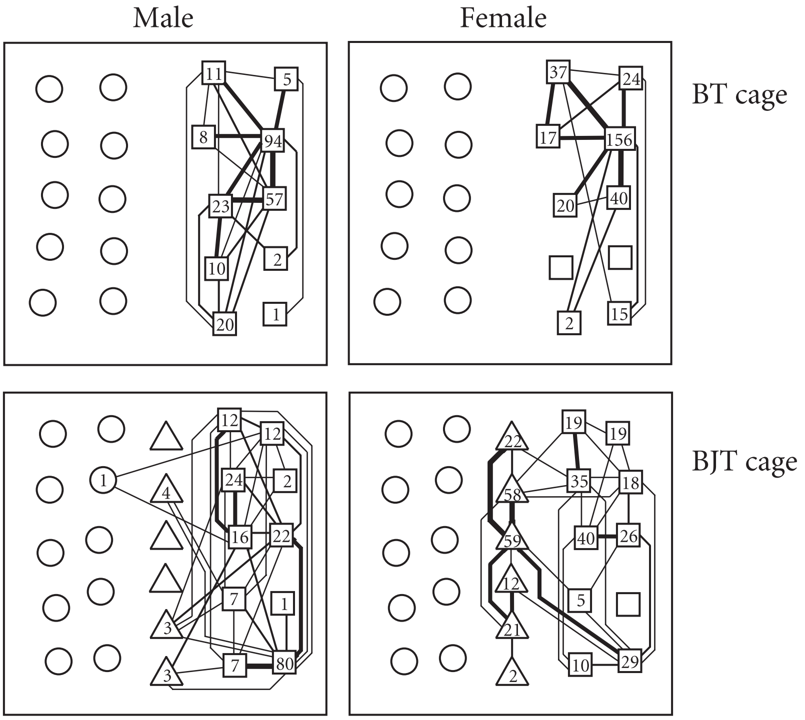

Figure 3 Distribution of adult beetles of *Henosepilachna niponica* in the field cages studied in 2000. Numerals indicate cumulative number of sightings of beetles on each plant. Where a beetle was successively observed on two different plants, the two plants are connected by a line. The thickness of line is approximately proportional to the frequency of beetle movement between the two plants.

Table 4 Number of egg masses laid by *Henosepilachna yasutomii* and *Henosepilachna niponica* on three or two plant species in the field cages

Cage and species of mother beetles	Plant			Total no. of egg masses
	Blue cohosh	Japanese nightshade	Thistle	
BT-Y (*H. yasutomii*)	142	–	0	142
BT-N (*H. niponica*)	0	–	161	161
BJT-Y (*H. yasutomii*)	107	17	0	124
BJT-N (*H. niponica*)	0	39	70	109

Discussion

The results of the present study are straightforward. When only their respective host plants were offered, almost all overwintered adults of *H. niponica* and *H. yasutomii* moved only between plants of their respective host species, even though the two host plants grew in close proximity (Table 2, Figures 2 and 3). Although a few individuals were found on the host plants of the other species, these seemed to be merely resting in an inactive state. Furthermore, the choice of host plants for oviposition was also very strict (Table 4). We found no eggs that were erroneously laid on plants of the other species. These results are consistent with previous results obtained under laboratory conditions (Katakura et al., 1989; Katakura & Hosogai, 1997) and indicate that there would be no error in host choice for food and oviposition in ecotones where blue cohosh and thistle occasionally grow side by side. As copulation occurs only on their own host plants (Table 3), the observed strict host fidelity could be a very strong reproductive barrier, preventing hybridization between the two beetle species under natural conditions.

However, such ecological isolation would be easily broken down when there is a common food plant (e.g., Japanese nightshade in the present study) that is eaten by both beetle species in addition to the two host species. As shown previously, we often observed the coexistence of *H. yasutomii* and *H. niponica* on the same plant of Japanese nightshade, where we also occasionally observed interspecific matings (Table 3). The consequence of this breakdown of the ecological barrier, when it happens, is not clear. However, lack of effective postmating barriers in this species pair suggests that the likely outcome is a merging of the two species through the production of fully viable and fertile F1 hybrids (Katakura et al., 1989; Katakura & Hosogai, 1997).

In fact, however, the merging of *H. niponica* and *H. yasutomii* has not occurred in nature. Their distribution ranges overlap in northern Japan, and in some cases they occur in very close proximity (Katakura, 1981; Katakura et al., 1989). Nevertheless, they exist as different biological entities, with distinct morphological (body size and elytral shape) as well as biological (host specificity) differences throughout their range of overlap. This suggests that cases where the presence of common host plants violates their ecological isolation due to host fidelity have been virtually not realized in nature. Alternatively, such a condition has occurred, but lasted for a very short time, and the trace of the resultant introgressive gene flow swept out through disruptive selection under the two-niche situation of the blue cohosh–thistle food resources. In any case, our study indicated that the fidelity to different host plants is enough to permit coexistence of *H. yasutomii* and *H. niponica* in nature. It must be mentioned, however, that our study suggested that reproductive isolation between *H. yasutomii* and *H. niponica* might be incomplete. For this, we must await detailed hybrid analyses of sympatric natural populations using reliable genetic markers. Also important would be a measurement of fitnesses of F_1 and later filial hybrids under natural conditions, although fitness reduction has not been noticed as far as the growth performance of the F_1 hybrids investigated under laboratory conditions is concerned (Katakura & Hosogai, 1994).

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