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ORIGINAL ARTICLE

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Adaptation to a new host plant, *Centrosema pubescens* (Fabales: Leguminosae), by the phytophagous ladybird beetle, *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae), in tropical Asia

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Abstract The host suitability of *Centrosema pubescens* (Leguminosae) was evaluated within two sympatric populations feeding on *Solanum* plants (Solanaceae) and *C. pubescens* in *Epilachna vigintioctopunctata* in Malaysia (Kuala Lumpur) and Indonesia (Bogor and Padang). In the Bogor and Padang populations, *Centrosema* strains had a significantly higher emergence rate than sympatric *Solanum* strains. In Kuala Lumpur, there was no significant difference in emergence rates between the two strains. When *Centrosema* strains from Kuala Lumpur and Padang were reared and maintained solely on *Solanum* plants, the emergence rate on *C. pubescens* gradually decreased with successive rearing generation and resulted in 0% in the 7th or 20th generations. These findings suggest that the current host suitability of *C. pubescens* depends on the previous experience of each population with the use of this plant as a host. However, we were not able to demonstrate from laboratory selection that *Solanum* strains increase the host adaptation to *C. pubescens* because every *Solanum* strain became extinct in the third generation when reared solely on *C. pubescens*.

Key words Host suitability · Host shift · Laboratory selection · Sympatric speciation

Introduction

Speciation is one of the great themes of evolutionary biology, and for many years it has been studied mainly from the point of view of the allopatric speciation involved in geographically isolated processes. Since the 1960s, sympatric speciation has been emphasized as an important process leading to genetic divergence in populations and new species formation (Bush and Smith 1998; Howard and Berlocher 1998). A host plant shift, the advance of herbivores into new plant species and subsequent host race formation, can be considered as one of the important factors leading to sympatric speciation, and it has been studied for many phytophagous insect species groups, e.g., *Colias* (butterfly) (Karowe 1990), *Yponomeuta* (small ermine moths) (Menken et al. 1992), *Ophraella* (leaf beetles) (Futuyma et al. 1995), and *Rhagoletis* (fruit flies) (Bush and Smith 1997). However, sympatric host race formation via host shift to new plant species has rarely been demonstrated by laboratory selection (Via 1990). For a herbivore, how many generations does it take for a new host race to be formed? How does the herbivore adapt to a new plant species, gradually or suddenly at a certain phase (generation)? These questions remain unanswered.

We have studied the sympatric speciation process based on differentiation in host plant utilization using the phytophagous Epilachninae beetle, *Epilachna vigintioctomaculata* Motschulsky, and related species groups distributed in cool temperate regions (Katakura et al. 1989; Katakura 1997). However, the *E. vigintioctomaculata* species group is an inappropriate model for laboratory selection for host suitability, because these species are univoltine with a long diapause period and because the wild host plants are limited in season. In contrast, *E. vigintioctopunctata* Fabricius, from tropical regions, is a suitable candidate for laboratory selection because this species is multivoltine without a diapause phase and because the host plants are available throughout the year (Shirai and Katakura 1999).

Although *E. vigintioctopunctata* feeds primarily on solanaceous plants, a leguminous weed, *Centrosema pubescens* Benth., was recently recorded as the host plant in Indonesia and Malaysia (Nishida et al. 1997). In Bogor, Indonesia, the population feeding on *C. pubescens* had a significantly smaller body size than the population feeding on *Solanum* plants such as *S. torvum*, *S. tuberosum*, and *S. melongena*. In the laboratory, adults of the former population preferred *C. pubescens* to *S. torvum* leaves, whereas the latter fed more on *S. torvum* than *C. pubescens* leaves.

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Table 1. Origin of *Epilachna vigintioctopunctata* populations examined in the present study

Strain[a]	Locality	Altitude (m)	Host plant in the field
S	Ulu Gadut, Padang, Indonesia	20	*Solanum torvum* (Solanaceae)
C	Limau Manis, Padang		*Centrosema pubescens* (Leguminosae)
S	Sukarami, Indonesia	930	*Solanum tuberosum*
S	Pasir Eurih, Bogor, Indonesia	265	*S. torvum*
C	Pasir Eurih, Bogor		*C. pubescens*
S	Jalan Ampang, Kuala Lumpur, Malaysia	17	*S. torvum*
C	Jalan Ampang, Kuala Lumpur		*C. pubescens*

[a] S, *Solanum*-feeding strain; C, *Centrosema pubescens*-feeding strain

Moreover, new adults that were reared on *C. pubescens* during the larval stage fed more on *C. pubescens* leaves than those reared on *S. torvum* (Nishida et al. 1997). From this difference in host preference, Nishida et al. (1997) have tentatively referred to the population feeding on *C. pubescens* as the "*Centrosema*-feeding strain (C-strain)" and the population feeding on *Solanum* plants as the "*Solanum*-feeding strain (S-strain)." *Centrosema pubescens*, native in South and Central America, is widely used as a plantation plant or as green manure in tropical regions worldwide (McIlroy 1972) and was introduced into Southeast Asia in the nineteenth century. Nishida et al. (1997) did not evaluate larval survival rate or egg production on *C. pubescens*; however, they reported a significant difference in host plant preference that suggests a sympatric host shift to a new plant species may have occurred in *E. vigintioctopunctata*, as in the case of other phytophagous insect species groups (Bush 1975; Futuyma and Peterson 1985; Bush and Smith 1997).

In the present study, three laboratory experiments were designed to evaluate the host suitability of *C. pubescens* for several tropical Asian populations of *E. vigintioctopunctata*. First, we compared the host suitability of *C. pubescens* within two sympatric strains (C- and S-strains) to establish whether the current suitability of *C. pubescens* depends on the previous experience of each population with the use of this plant as a host. Second, we monitored processes that the larval performance on *C. pubescens* increases in response to laboratory selection in which the S-strain was reared and maintained solely on *C. pubescens* leaves. Third, the change in the larval performance on *C. pubescens* was followed when the C-strain was reared and maintained solely on *Solanum* plants isolated from *C. pubescens*. Both the second and third experiments could indicate the rate of change in the host suitability of *C. pubescens*. In addition, the third experiment can support the verification provided by the first experiment.

Material and methods

Insect collection

Two sympatric populations (S- and C-strains) were collected from *S. torvum* and *C. pubescens* in Kuala Lumpur (Malaysia), Padang, and Bogor (Indonesia). The two strain localities were approximately 5 km (Padang), 1 km (Bogor), and 0.5 km distant (Kuala Lumpur), respectively (Table 1). Both Bogor strains were collected from the same study sites as reported in Nishida et al. (1997). In Kuala Lumpur, no other host plants of *E. vigintioctopunctata* were found between the collection sites. In Bogor and Padang, *S. torvum*, other *Solanum* plants, and *C. pubescens* grew sporadically in the two sites. In the Sukarami highlands approximately 30 km east of Padang, where *E. vigintioctopunctata* was not found on *C. pubescens*, beetles were collected only from potatoes.

Experiment 1: Host suitability of C. pubescens within sympatric populations

For each local population, 20 or 25 pairs from the collected adults were introduced into a mesh cage ($30 \times 35 \times 50$ cm high) with a potted black nightshade, *Solanum nigrum* L. Egg masses laid on *S. nigrum* leaves were taken, and six hatched larvae were transferred to a plastic dish (9 cm in diameter, 4.5 cm deep). Larvae were reared on the young leaves of *C. pubescens*. A fresh leaf was added to the dish every 1 or 2 days until the third stadium and daily during the fourth stadium. After emergence, the pronotal width measurement was determined for the new adults.

Experiment 2: Maintenance of S-strain on *C. pubescens*

New adults of the S-strain from Bogor and Kuala Lumpur that emerged in the foregoing larval experiments were used. One female and two males were transferred to a plastic dish (9 cm in diameter, 4.5 cm deep), and a fresh *C. pubescens* leaf was added daily to obtain eggs. Hatched larvae were reared on a *C. pubescens* leaf in the same manner as described in the earlier experiment.

Experiment 3: Maintenance of C-strain solely on the *Solanum* plant

Among the offspring of the C-strain that had been reared and maintained solely on *S. nigrum*, the emergence rate on *C. pubescens* was monitored in the 5th and 7th generations for the Kuala Lumpur population, and in the 4th, 12th, 15th,

and 20th generations for the Padang population. For each sample, egg masses laid on *S. nigrum* leaves were randomly collected, and hatched larvae from one egg mass were divided onto the *S. nigrum* and *C. pubescens* leaves. Larval rearing was done in the same manner as described. Rearing for successive generations on *S. nigrum* was conducted as follows. Thirty pairs of females and males were introduced into a mesh cage with potted *S. nigrum*. For 2 days between 20 and 25 days after emergence, when females most frequently oviposited, a total of 30 egg masses were collected and transferred to another cage. Hatched larvae were then reared on potted *S. nigrum*, and new plants were added daily during the third and fourth stadium, while removing the plants exhausted by the larvae. After the emergence of new adults, 30 females and 30 males were randomly chosen as the parental stock of the next generation.

All laboratory studies were conducted at the National Institute of Agro-Environmental Sciences (NIAES), Tsukuba, under 26°C and 14L:10D conditions. *Centrosema pubescens* and *Solanum nigrum* were grown in the greenhouse. Voucher specimens were deposited in the Laboratory of Insect Systematics, NIAES.

Results

Experiment 1

Table 2 shows the survival rate and developmental period in the immature stage, the sex ratio of the emerged adults, and the adult body size when reared on *C. pubescens* leaves. In Padang populations, the C-strain had a significantly higher emergence rate than S-strains. The developmental period and adult body size were not statistically tested between the two strains because the S-strain produced only one new adult. The S-strain of Sukarami had as low an emergence rate (1.4%) as the S-strain of Padang. In the Bogor populations, the C-strains had a significantly higher emergence rate and a smaller body size than the S-strains. The developmental period and sex ratio of new adults did not differ between the two strains from Bogor. In Kuala Lumpur, the C-strain showed a significant smaller body size than the S-strain, but there were no significant differences in the emergence rate, developmental periods, or sex ratio of new adults.

In all populations, irrespective of strain, 80%–100% of hatched larvae fed on *C. pubescens* leaves and 50%–90% of the larvae reached the second stadium. However, except for the C-strain of Bogor, in the remaining six samples the larvae gradually died, resulting in low emergence rates of 1.4%–36.1% (Table 2).

Experiment 2

Table 3 shows the consequences of rearing successive generations of S-strains solely on *C. pubescens* leaves. Padang and Sukarami could not produce a second generation because no female was generated from the first rearing on *C. pubescens* leaves. The Bogor population showed an increased emergence rate (59.7%) in the second generation,

Table 2. Larval survival and development when reared on *Centrosema pubescens* leaves in the *E. vigintioctopunctata* populations

Locality	Strain	Survival rate (%)[a]			Developmental[a] period from L1 to adult (days)	Sex ratio of emerged adults (♀/[♀ + ♂])	Pronotal widths of adults (mm)[a]	
		L2	L4	Adult			Female	Male
Padang	S	48.6	11.1	1.4 ± 4.8 (12)*	23 (1)	0.0 (0/1)	— (0)	2.45 (1)
	C	85.7	51.2	36.1 ± 28.3 (12)	24.6 ± 1.1 (11)	0.31 (8/26)	2.81 ± 0.24 (8)	2.66 ± 0.18 (18)
Sukarami	S	75.0	1.4	1.4 ± 4.8 (12)	31 (1)	0.0 (0/1)	— (0)	2.60 (1)
Bogor	S	88.9	58.3	34.7 ± 39.2 (12)*	24.8 ± 1.9 (6)	0.24 (6/25)	3.03 ± 0.15 (6)**	2.74 ± 0.18 (19)**
	C	100.0	100.0	75.0 ± 25.1 (12)	23.8 ± 1.8 (12)	0.32 (17/54)	2.65 ± 0.19 (14)	2.43 ± 0.20 (33)
Kuala Lumpur	S	77.4	50.0	34.7 ± 20.7 (12)	26.7 ± 2.6 (11)	0.52 (13/25)	2.93 ± 0.20 (13)*	2.77 ± 0.17 (11)*
	C	83.3	40.3	22.2 ± 17.9 (12)	25.2 ± 1.6 (9)	0.44 (7/16)	2.54 ± 0.14 (6)	2.55 ± 0.11 (5)

[a] Mean ± SD (no. of replications)
*,** Significant difference between strains in the same locality by Mann–Whitney *U*-test (* $P < 0.05$; ** $P < 0.01$)

Table 3. Consequences of rearing for successive generations on *C. pubescens* leaves in the *Solanum*-feeding strains

Locality	Rearing generation	Survival rate[a] from L1 to adults (%)	Sex ratio of emerged adults (♀/[♀ + ♂])	No. females that laid fertilized eggs
Padang	First	1.4 ± 4.8 (12)	0.0 (0/1)	0
Sukarami	First	1.4 ± 4.8 (12)	0.0 (0/1)	0
Bogor	First	34.7 ± 39.2 (12)	0.24 (6/25)	3
	Second	59.7 ± 24.0 (12)	0.42 (18/43)	3
	Third	11.1 ± 13.0 (12)	0.38 (3/8)	0
Kuala Lumpur	First	34.7 ± 20.7 (12)	0.52 (13/25)	8
	Second	30.3 ± 26.7 (11)	0.35 (7/20)	3
	Third	7.2 ± 10.8 (14)	0.0 (0/6)	0

[a] Mean ± SD (no. of replications)

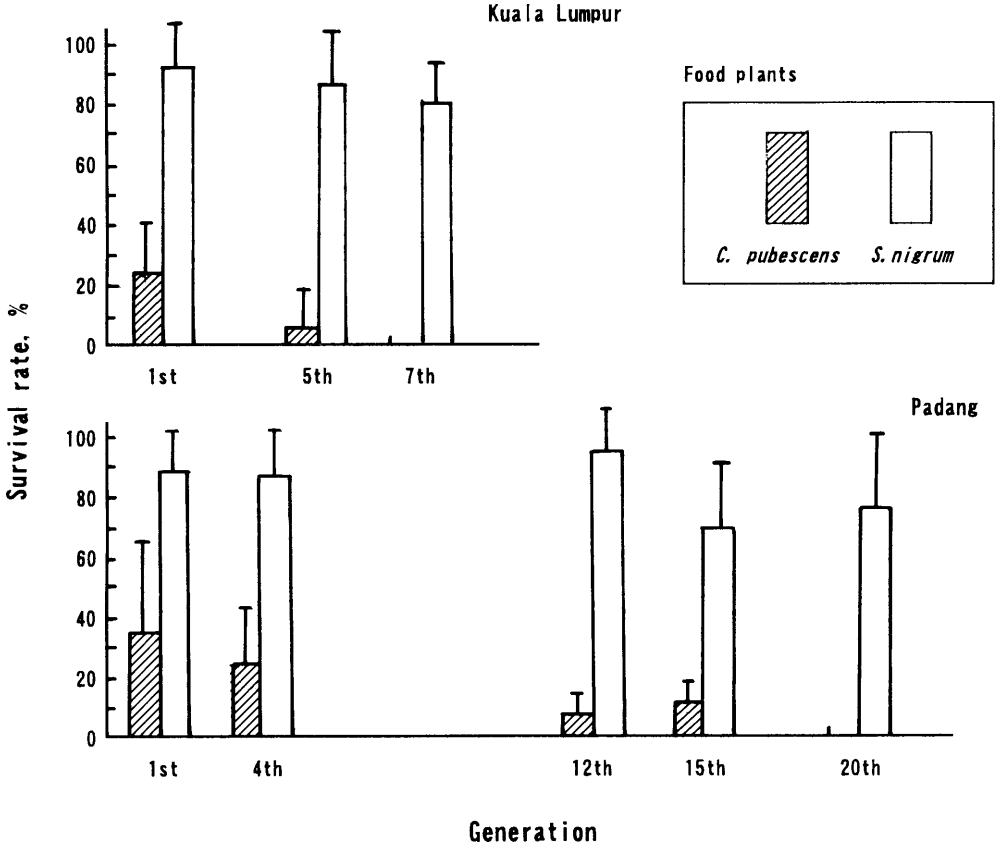

Fig. 1. Changes in larval survival rate (mean ± SD) of the *Centrosema*-feeding strains in Kuala Lumpur (*upper graphs*) and Padang (*lower graph*) when successively maintained on *Solanum nigrum*

but the rate decreased in the third generation (11.1%), and a fourth generation was not produced because none of the eight females from the third generation oviposited eggs on *C. pubescens* leaves. The Kuala Lumpur population showed a gradually decreasing emergence rate with subsequent generations and could not produce a fourth generation because there were no new females in the third generation. In both the Bogor and Kuala Lumpur populations, throughout the generations reared, the females tended to live for a shorter period and fewer than half the females laid fertilized eggs (Table 3).

Thus, none of the S-strains was able to be maintained solely on *C. pubescens* for more than three generations because of a low larval survival rate, a male-biased sex ratio in the new adults, and low fecundity in the new females.

Experiment 3

Figure 1 shows the change in the emergence rate on *C. pubescens* when C-strains of Kuala Lumpur and Padang had been reared and maintained on *S. nigrum*. The Kuala Lumpur population showed a decreased emergence rate (4.2%) in the 5th generation, with the rate dropping to 0% in the 7th generation. Padang also showed a gradually decreased emergence rate in subsequent generations, with the rate falling to 0% in the 20th generation. The two populations had a relatively high emergence rate, 70%–90%, throughout the generations, when assayed on *S. nigrum* leaves.

Discussion

Host suitability between sympatric populations

In the two sympatric strains in Bogor, C-strains showed a significantly higher emergence rate and smaller adult body size on *C. pubescens* leaves than did the S-strains (see Table 2). These results supported those of Nishida et al. (1997). Among the seven local populations tested, the C-strain from Bogor showed the highest emergence rate of 75% (Table 2), and this strain significantly preferred *C. pubescens* to *Solanum* leaves in the experiment reported by Nishida et al. (1997). Thus, the laboratory results are likely to reflect a difference in the plant species that each population uses as a host in the field. However, the S-strain also showed a relatively high emergence rate, 34.7%. The two strains in Bogor were approximately 1 km distant from each other, and no reproductive isolation was observed between the strains (Nishida et al. 1997). In Bogor, it seemed that two strains have not entirely diverged, and adult beetles may move to some extent between each vegetation (*C. pubescens* and solanaceous plants).

In Padang, the C-strains showed a significantly higher emergence rate on *C. pubescens* leaves than did the S-

strains (Table 2). Both the S-strains, in Padang and in Sukarami approximately 30 km east of Padang, showed a very low emergence rate of 1.4% on *C. pubescens* leaves. Moreover, when the C-strain of Padang had been isolated from *C. pubescens*, the emergence rate on *C. pubescens* gradually decreased and then fell to 0% in the 20th generation (see Fig. 1). The results from Padang and Sukarami also suggest that the host suitability of *C. pubescens* depends on the host plant species that each local population uses in the field, as in Bogor. Therefore experiment 1 showed that the two Indonesian populations (Bogor and Padang) are appropriate candidates for future study on sympatric speciation via host plant shift.

In contrast, the two strains in Kuala Lumpur did not exhibit a significant difference in their host suitability. These two strains were located within 0.5 km of each other. Adult beetles may move frequently between the two patches, although no other host plants were found between the two collection sites. At present, we have concluded that these local populations do not include a "host plant strain."

Evaluation of host suitability by laboratory selection

Experiment 3 revealed that the larval performance on *C. pubescens* has been gradually decreased through isolation from *C. pubescens*, and fell to 0% in the 7th generation in Kuala Lumpur, or in the 20th generation in Padang; the approximate rate of decrease in the emergence rate per generation was 3.7 and 1.9, respectively. These generation times (7th and 20th) correspond to about 1 to 3 years in the field, because *E. vigintioctopunctata* produces eight generations a year on *S. torvum* in Bogor (Nakamura et al. 1990) and on *C. pubescens* in Padang (I. Abbas and K. Nakamura, personal communication). It was not as well known how phytophagous insects lost host plant suitability when they were isolated from new host plants (Via 1990). The present experiment demonstrated that *E. vigintioctopunctata* gradually lost larval performance on *C. pubescens* within relatively few generations (Fig. 1).

In the present study, we focused most intensely on experiment 2, but were unable to demonstrate from laboratory selection that the S-strain of *E. vigintioctopunctata* promotes larval performance on *C. pubescens* in response to successive rearing on *C. pubescens* (see Table 3). We know that *E. vigintioctopunctata* can be maintained by laboratory rearing for many generations because this species has been successfully reared over 20 generations on *S. nigrum* plants (see Fig. 1). The failure in successive rearing on *C. pubescens* can be attributed to a low larval survival rate, a male-biased sex ratio in new adults, and low fecundity in new females. These findings may imply an influence of inbreeding from small population size. However, we thought that other factors than inbreeding may prevent successive rearing on *C. pubescens* in the laboratory, because three tropical *Epilachna* species (*E. vigintioctopunctata*, *E. pusillanima* Mulsant, and *E. pytho* Mulsant) were easily maintained on *S. nigrum* or pumpkin leaves for more than 2 years even by using a small parental stock of four or five pairs (Y. Shirai, unpublished data). In the present experiment, a slight qualitative deterioration may occur in the *C. pubescens* leaves grown in the greenhouse, because larvae of *E. vigintioctopunctata* refused to eat slightly hardened or withered leaves of *C. pubescens* in the experiment conducted at Indonesia (T. Nishida, personal communication).

Laboratory selection has been conducted numerous times for studies on host plant use evolution by phytophagous insects (Futuyma and Peterson 1985; Via 1990; Rice and Hostert 1993). However, a rapid response to selection for host plant use has been demonstrated only in the cases of the Hessian fly (Gallun et al. 1961), the two-spotted spider mite (Gould 1979), and the brown rice planthopper (Claridge and den Hollander 1982; Pathak and Heinrichs 1982). Each of these studies showed a rapid adaptation to a plant variety resistance to insect pests within a single plant species. Thus, none of the laboratory selection studies has demonstrated a successful host shift between different plant species. To demonstrate changes in the host suitability of *C. pubescens* for *E. vigintioctopunctata*, further experiments should be conducted using field cages in tropical areas, as was done in the studies of Passifloraceae and *Heliconius* butterflies (Jiggins et al. 1997).

Advantage on new host plant, *C. pubescens*

S-strains in Indonesia have been studied in both the field and the laboratory (Nakamura et al. 1990), although there has been little study of C-strains. Wild C-strain adults have a significantly smaller body size than S-strain adults (Nishida et al. 1997), and the females produce only 18–80 eggs when reared on *C. pubescens* (Shirai and Katakura 1999). This level of egg production is much lower than that of females on *S. torvum* (427–771) (Abbas et al. 1985; Nakamura et al. 1995). Thus *C. pubescens* is a less suitable host plant in terms of reproduction and larval development than *Solanum* plants. Why does *E. vigintioctopunctata* shift to *C. pubescens* from *Solanum* plants? Does *C. pubescens* give any ecological advantage to these beetles? Fragmentary field research has suggested three possibilities: (1) *C. pubescens* has a larger biomass per patch than *S. torvum*; (2) predators such as ants (*Anoplalepis* sp.) are less abundant on *C. pubescens*; and (3) *C. pubescens* has more fresh leaves than *S. torvum*, even in dry seasons (S. Nakano and T. Nishida, personal communication). Do these factors really accelerate the host shift from *S. torvum* to *C. pubescens*? Future field studies are needed to evaluate the mortality factors of *E. vigintioctopunctata* populations on *C. pubescens* and *S. torvum*, the host plant phenology in the dry and rainy seasons, and the dispersal movement of adult beetles between *C. pubescens* and *Solanum* plant vegetation.

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