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[REVIEW]

Species of *Epilachna* Ladybird Beetles

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ABSTRACT—The present paper reviews current studies on various aspects of reproductive isolation in a series of closely related phytophagous ladybird beetles called the *Epilachna vigintioctomaculata* complex, which is composed of two groups and four species: group A comprising *E. vigintioctomaculata*, and group B comprising one northerly distributed species, *E. pustulosa*, and two southerly distributed species, *E. niponica* and *E. yasutomii*.

Being broadly sympatric from middle through northern Japan, the two groups are reproductively isolated by a combination of several factors, each of which functions as an incomplete barrier to gene flow: difference of host plants, weak sexual isolation, and low hatching rates of eggs produced by interspecific matings. Conspecific sperm precedence further lessens the probability of producing hybrids in mixed populations. By contrast, the two sympatric species of group B, *E. niponica* and *E. yasutomii*, are reproductively isolated from each other solely by fidelity to different host plants. However, there are no effective barriers to gene exchange between either of these two species and the northerly distributed *E. pustulosa*.

These situations have relevance to various controversial issues in evolutionary biology, covering the mode of speciation in host specific insects, role of postinsemination barriers to fertilization, reinforcement of reproductive isolation, and treatment of allopatric populations in speciation studies. The *E. vigintioctomaculata* complex thus offers a rare opportunity to extend our understanding of the nature of animal species and their origin.

INTRODUCTION

Species is a basic category of biological classification, and it is often regarded as the most fundamental to all fields of biological sciences. Yet there is no general consensus among evolutionary biologists about what is a species. One widely accepted view, known as the "biological species concept", regards a species as a genetically isolated unit (Dobzhansky, 1937; Mayr, 1942, 1963); it is the members in aggregate of a group of populations that interbreed or potentially interbreed with each other under natural conditions (Futuyma, 1986). However, there are many other definitions of species (for a review, see Endler, 1989; Akimoto, 1992).

Speciation, the origin of a new species, has also been a subject of heated arguments (Otte and Endler, 1989; Coyne, 1992). Most evolutionary biologists believe that allopatric speciation, speciation following splitting of the ancestral population by extrinsic (geographic) barriers, is the most prevailing mode of speciation in animals (Mayr, 1963; Futuyma and Mayer, 1980; Futuyma, 1986). Intrinsic barriers to gene flow are thought to evolve as accidental byproducts of divergence in allopatry, but whether such barriers can be reinforced at the zone of secondary contact of two cognate species or not is controversial (Dobzhansky, 1940; Butlin, 1987, 1989; Coyne and Orr, 1989; Howard, 1993). Others believe, moreover, that intrinsic barriers to gene flow evolve within a population (in sympatry) or in an adjacent population (parapatry) despite gene flow (Bush, 1969, 1975, 1994; Endler, 1977; White, 1978; Diehl and Bush, 1984; Barton and Hewitt, 1985; Bush and Howard, 1986; Tauber and Tauber, 1989).

To better understand the real nature of species and their origin, therefore, careful investigations of the natural populations of various organisms are still indispensable. It would be particularly fruitful to investigate mating barriers in coexisting taxa that are closely related, since almost all the issues in these contentious areas of evolutionary biology concern reproductive isolation and its evolution. The purpose of the present paper is to review current studies on the various aspects of reproductive isolation in a series of closely related phytophagous ladybird beetles called the *Epilachna vigintioctomaculata* species complex (Epilachninae, Coccinellidae).

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The *Epilachna vigintioctomaculata* complex

The *Epilachna vigintioctomaculata* complex differs greatly in external morphology and food plants in and around the Japanese Archipelago. This species complex has been the subject of intensive studies since 1940's by researchers of diverse fields of evolutionary biology, in particular those who have a specific interest in speciation (Watanabe and Sakagami, 1948; Ehara, 1952, 1953; Yasutomi, 1954; Iwao, 1959; Watanabe and Suzuki, 1965; Kuboki, 1978; Katakura, 1981a, 1988; Katakura *et al.*, 1981, 1989; Nakano, 1981, 1985; Hoshikawa, 1983a, b, 1984; Tsurusaki *et al.*, 1993; Katakura and Hosogai, 1994, 1997).

Based on various sorts of information covering morphology, food habits and their geographic variations, Katakura (1981a) classified the *E. vigintioctomaculata* complex into two groups and four species (Table 1, Fig. 1): group A, *E. vigintioctomaculata* Motschulsky; group B, *E. pustulosa* Kôno, *E. niponica* Lewis, *E. yasutomii* (Katakura). The separation of the species complex into two groups is based primarily on morphological evidence and the difference of host plants (Katakura, 1981a). Adults are discriminated by subtle but relatively stable differences in a number of characteristics such as elytral spot patterns, antennal segments and the genitalia of both sexes. Furthermore, group A occurs on solanaceous and cucurbitaceous plants while the members of group B usually occur on thistle (*Cirsium* spp.) and/or blue cohosh (*Caulophyllum robustum*). The dichotomy has recently been supported by karyological evidence (Tsurusaki *et al.*, 1993) and analyses of mitochondrial DNA (N. Kobayashi *et al.*, unpubl. data).

On the other hand, recognition of the three species of group B is operational, and is mainly based on biological evidence and distributional information (Katakura, 1981a). Recent analyses of the cytochrome oxidaze I gene of mitochondrial DNA clarified that the three species are very closely related genetically (N. Kobayashi *et al.*, unpubl. data). The difference between the populations of group B was 0.002 to 0.005 /bp, nearly equivalent to that found among the individuals of the same population. This result implies that the morphological and biological divergence of group B occurred rapidly over a very short geological time.

Life history and modes of reproduction

Various factors can act as intrinsic barriers to gene flow between sympatric taxa (Mayr, 1963; Dobzhansky, 1970; Littlejohn, 1981). These barriers are classified into those that operate before mating (=premating) and after mating (postmating). Since life history traits and modes of reproduction often function as important premating barriers, those of the members of the *E. vigintioctomaculata* complex are summarized here before describing the reproductive isolation of selected pairs.

1) *Life cycle*: All members of the *E. vigintioctomaculata* complex are univoltine and hibernate as adults (Katakura, 1981a). New adults emerge in mid summer to early fall, and enter hibernation by late fall in a litter in or near their habitats. Most overwintered adults die by summer but some enter a second hibernation (Nakamura and Ohgushi, 1979).

2) *Oviposition and mating activities*: Usually only overwintered females oviposit (Katakura, 1976). On the other hand,

Table 1. Composition of the *Epilachna vigintioctomaculata* species complex, with geographic distribution and variation of component species

Group and species	Distribution	Geographic variation in	
		external morphology	host plants
Group A			
E. vigintioctomaculata	Cool temperate regions of Eastern Asia; in Japan, occurring in Hokkaido and cool parts of Honshu, Shikoku, Kyushu	Less conspicuous, with three geographic forms (V-I to III) in and around Japan	Slight; usually on potato (Solanaceae) but in Hokkaido also on *Schizopepon bryoniaefolius* (Cucurbitaceae)
Group B	Endemic to cooler parts of Japan (Hokkaido, Honshu)	Variable according to species	Feeding on thistle (Compositae) and/or blue cohosh (Berberidaceae), but variable according to species
E. pustulosa	Hokkaido except for the eastern part and Oshima Peninsula	Conspicuous with four distinct forms (P-I to III, III')	Less conspicuous; usually on thistle; one geographic form (P-III) also on blue cohosh
E. niponica	Oshima Pen. of Hokkaido, Honshu	Conspicuous with at least three forms (N-I to III)	Not conspicuous; exclusively on thistle
E. yasutomii	Oshima Pen. and eastern part of Hokkaido, Honshu (Tohoku to Chubu districts)	No variation (except for size) detectable	Conspicuous; usually on blue cohosh; some populations subsidiarily on *Chelidonium japonicum* (Papaveraceae) and/or *Panax japonicus* (Araliaceae); some on *Scopolia japonica* (Solanaceae) or on potato (Western Tokyo form *Epilachna*)

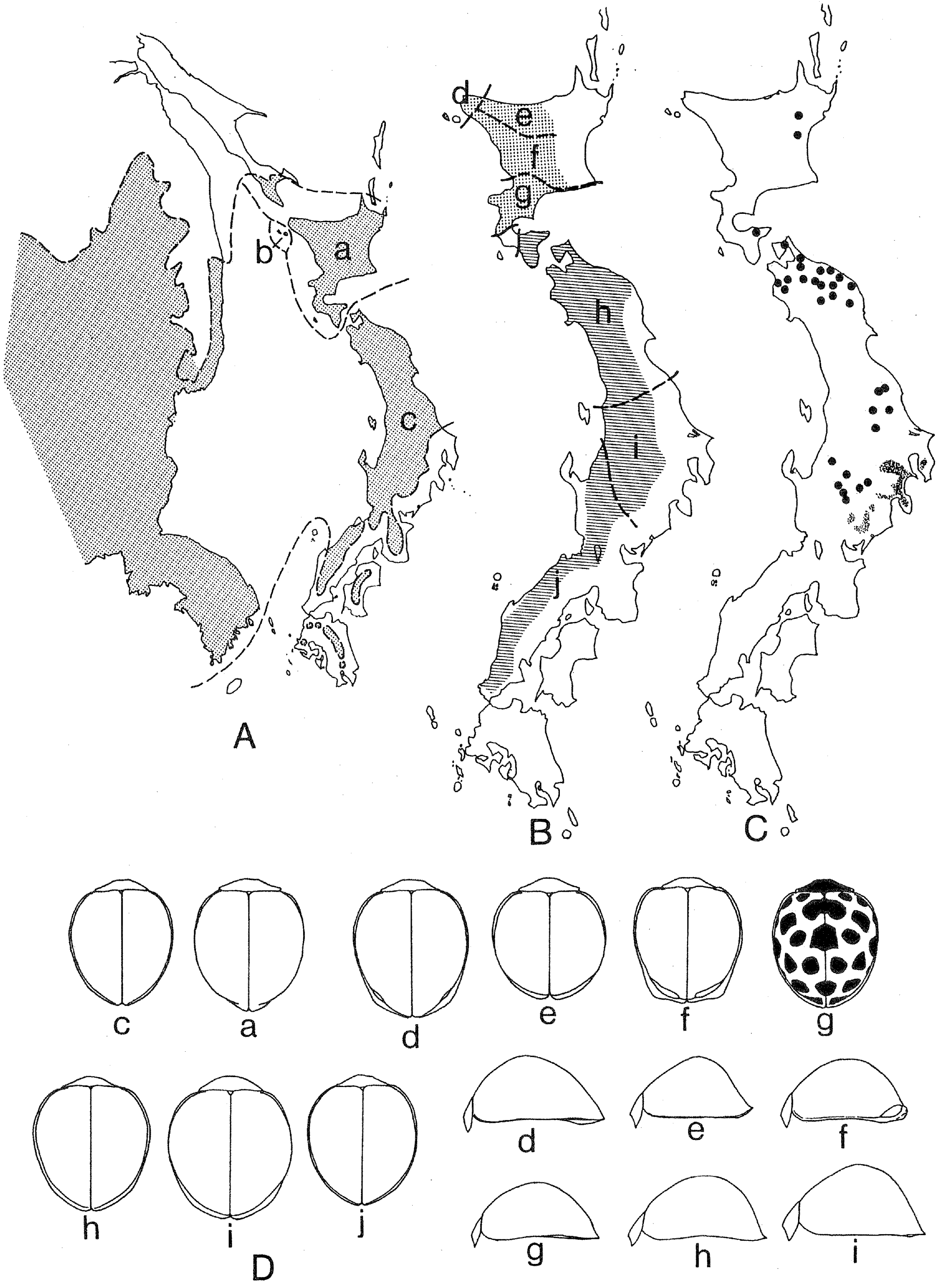

Fig. 1. Geographic distribution (**A** - **C**) and variation of elytral shape (**D**) (dorsal view for a, c, d - j; lateral view for d - i; markings on pronotum and elytra are omitted except the dorsal view of g) of the members of the *Epilachna vigintioctomaculata* complex (based on Katakura, 1988). (**A**) *E. vigintioctomaculata* (a - c); (**B**) *E. pustulosa* (d - g) and *E. niponica* (h - j); (**C**) *E. yasutomii* (the range of the so-called "Western Tokyo form" is shaded). a - c, V-I to V-III; e - g, P-I to III; d, P-III'; h - j, N-I to III.

both males and females mate repeatedly during their lives (Nakano, 1987), from the summer of emergence to just before death (in most cases by the mid summer of the next year) with the intervention of winter dormancy. They usually mate on their host plants. More than half of newly emerging females of *E. vigintioctomaculata* and *E. pustulosa* mate before entering hibernation (Katakura, 1982).

3) *Sperm storage and sperm longevity*: After copulation, females keep spermatozoa in a pair of lateral swellings located at the common oviduct, a structure thus far known only in epilachnine beetles (Dobzhansky, 1924; Katakura, 1981b; Katakura *et al.*, 1994). Spermatozoa kept by females are viable and fertile for a few months in laboratory conditions. In natural conditions, too, stored spermatozoa seem long-lived (Katakura, 1982).

Reproductive isolation between the two groups of the *E. vigintioctomaculata* complex

The two groups co-occur in most parts of Hokkaido and Honshu, usually on different host plants. Reproductive isolation has been studied in detail between *E. vigintioctomaculata* and *E. pustulosa* in Sapporo and the vicinity as a representative case of the sympatry of the two groups (Katakura, 1976, 1981a, 1982, 1986a, b; Katakura and Nakano, 1979; Katakura and Sobu, 1986; Nakano, 1981, 1985, 1987; Tsurusaki *et al.*, 1993). Various factors function as barriers to gene flow between the two species (Table 2).

1) *Ecological and seasonal isolation*: *Epilachna vigintioctomaculata* is known as a pest of potato plants. In Hokkaido, it also occurs on a wild cucurbit, *Schizopepon bryoniaefolius* (Katakura, 1975). On the other hand, *E. pustulosa* in the Sapporo area occurs mainly on thistle (mostly *Cirsium kamtschaticum*) and blue cohosh. This difference in the main "host plants", here defined as the plants on which larvae complete their growth under natural conditions, reduces the gene flow between the two species to some extent. Furthermore, the difference of host plants seems to result in the phenological difference of the two ladybird beetle species (Katakura, 1976): posthibernating adults of *E. pustulosa* appear in late April to early May whereas those of *E. vigintioctomaculata* in late May to early June, coinciding with the sprouting of the respective host plants.

However, isolation by host specificity is not complete (Table 3; Katakura, 1981a). The main host plant of one species may be a subsidiary host plant or an edible plant (a plant occasionally eaten by adults or larvae but not used as a host) of the other species. Moreover, there are many plants, mostly cultivated plants and weeds, used as edible plants by both species. The coexistence of *E. vigintioctomaculata* and *E. pustulosa* is occasionally found on these plants.

2) *Sexual isolation*: When given choices between the two species of females under laboratory conditions, males tend to choose conspecifics (Katakura and Nakano, 1979). However, interspecific matings are not rare under such conditions. In natural conditions as well, occasional interspecific matings are known. In a mixed population of the two species which was found occurring on *S. megacarpum* in the vicinity of Sapporo, interspecific matings were rather common, totally 38.7% of the matings observed during 1978–1980 (N=223) (Nakano, 1987).

3) *Gametic isolation*: The slight difference in genitalia between the two species (Katakura, 1981a) does not function as a barrier to gene flow. Insemination is successful and normal in interspecific matings. However, approximately 75 - 90% of spermatozoa inseminated in heterospecific females are lost in the course of sperm migration from the bursa copulatrix to the sperm storage organ (Katakura, 1986a), possibly owing

Table 2. Effects of various factors (isolating mechanisms) on the reproductive isolation between *E. vigintioctomaculata* (Ev) and *E. pustulosa* (Ep)

Factor	Effect	Notes
Premating		
Ecological isolation	Strong	Difference in host plants (see Table 3).
Seasonal isolation	Weak	Both species overwinter as adults; Ep appears approximately one month earlier than Ev.
Sexual (behavioral) isolation	Weak	Weak but statistically significant positive choice by males.
Mechanical isolation	None	No significant difference in genitalic structure; sperm transfer between Ep and Ev normal.
Postmating (prezygotic)		
Gametic isolation	Strong	Distinctly fewer number of heterospecific spermatozoa in female sperm reservoir; only a few percent of eggs produced by hybridization hatch (owing to the failure of fertilization and death of embryos; see note for inviability of hybrid embryos).
Postmating (postzygotic)		
Inviability of hybrid embryos	Strong	Death of hybrid embryos might be a delayed effect of gametic isolation (by fertilizing with a deteriorated heterospecific spermatozoon), or due to subtle karyological difference of Ep and Ev.
Inviability of hybrids after hatching	None	Hybrid larvae are as viable as normal Ep and Ev larvae.
Hybrid sterility	None?	Hybrids are fertile; hatching rates of eggs laid by the hybrids might be somewhat lower than those of normal Ep or Ev eggs but are higher than those of eggs produced by interspecific crossings.

Table 3. A list of host plants (plants on which larvae complete the growth under natural conditions) and edible plants (plants occasionally eaten by adults or larvae) of *E. vigintioctomaculata* (Ev) and *E. pustulosa* (Ep) confirmed under natural conditions in the Sapporo area (modified from Katakura, 1981a)

Plant family and species		Ev	Ep
Chenopodiaceae	spinach *Spinacia oleracea*		+
Berberidaceae	blue cohosh *Caulophyllum robustum*		+++
Cruciferae	Chinese cabbage *Brassica campestris*	+	+
Leguminosae	soy bean *Glycine max*	+	
	scarlet bean *Phaseolus coccineus*	+	
	yard long bean *Vigna Catiang* var. *sinensis*	+	+
Buxaceae	*Pachysandra terminalis*		+
Araliaceae	sennoki *Kalopanax pictus*		+
Solanaceae	*Physaliastrum japonicum*	+	
	black nightshade *Solanum nigrum*	++?	+
	S. megacarpum	+++	++
	potato *S. tuberosum*	+++	++?
	egg plant *S. melongena*	+	+
	tomato *Lycopersicon esculentum*	+	+
	green pepper *Capsium annuum*	+	+
	Datura stramonium var. *chalybea*	+	
Cucurbitaceae	*Schizopepon bryoniaefolius*	+++	+
	water melon *Citrullus vulgaris*	+	
	cucumber *Cucumis sativus*	+	+
	melon *C. melo*	+	
	pumpkin *Cucurbita pepo*	+	
Compositae	thistle *Cirsium* spp.		+++
	creeping thistle *Breea setosa*	+	++
	great burdock *Arctium Lappa*	+	+

+++ main host plant; ++ subsidiary host plant; + edible plant; ? assumed.

Table 4. Hatching rates of eggs (%, mean ± SD, the number of replicates in parentheses) produced by various combinations of matings involving the two groups of the *E. vigintioctomaculata* complex. Group A: V, *E. vigintioctomaculata*. Group B: P, *E. pustulosa*; N, *E. niponica*; Y, *E. yasutomii*; WT, "Western Tokyo form *Epilachna*" (= *E. yasutomii*).

Combination of crosses		Conspecific mating		Interspecific mating		Source
Group A	Group B	A♀×A♂	B♀×B♂	A♀×B♂	B♀×A♂	
V	P	71.6±11.0 (9)	74.2±10.9 (10)	4.0±11.0 (7)	4.0±6.7 (10)	Katakura and Nakano (1979)
V	P	50.9±18.6 (12)	60.0±18.3 (8)	5.3± 9.8 (11)	0.6±0.9 (8)	Katakura (1986a)
V	P	82.3±10.4 (13)	66.7±20.5 (16)	24.3±25.3 (14)	1.3±1.9 (15)	Katakura and Sobu (1986)
V	N	62.6±15.4 (9)	25.8±20.3 (9)	7.5± 7.6 (9)	1.9±4.2 (9)	Katakura, unpubl.
V	Y	62.6±15.4 (9)	40.8±25.3 (7)	4.5± 8.4 (10)	2.1±2.5 (6)	Katakura, unpubl.
V	Y	82.4± 7.1 (2)	94.6± 0.7 (2)	13.1± 7.6 (5)	–	Tomioka (1988)
V	WT	81.4± 7.8 (9)	65.4±10.3 (10)	22.4±22.6 (6)	1.7±3.7 (8)	Katakura, unpubl.
V	WT	70.2±22.7 (10)	55.3±17.6 (7)	1.8± 2.2 (4)	2.6±4.5 (13)	Nakano (1987)

to the incompatibility between the spermatozoa and genital tract of the females.

The spermatozoa kept in the sperm reservoir of heterospecific females are motile, although they often appear to be somewhat anomalous compared with those in conspecific females (Katakura, 1986a).

4) *Low hatching rates of eggs produced by hybridization*: Hatching rates of eggs produced by interspecific matings are much lower than those by intraspecific matings, though results are variable according to experiments (Katakura and Nakano, 1979; Nakano, 1985; Katakura, 1986a; Katakura and Sobu, 1986). Some examples are given in Table 4. This low hatchability is caused by the failure of fertilization and death of hybrid embryos (Fig. 2; Katakura and Sobu, 1986).

The failure of fertilization can be explained by the gametic isilation mentioned above. Spermatozoa kept by heterospecific females may be too few to fertilize every egg, or they may have suffered a reduction of fertility in the genital tract of the females.

On the other hand, at least three explanations are possible for the death of hybrid embryos. First, the substantially high mortality of hybrid embryos may involve the death due to direct interaction of incompatible gene systems coexisted in the hybrid individual, a set of genes from the mother and the other from the father (Dobzhansky, 1970). Secondly, the death of hybrid embryos may be a delayed effect of gametic isola-

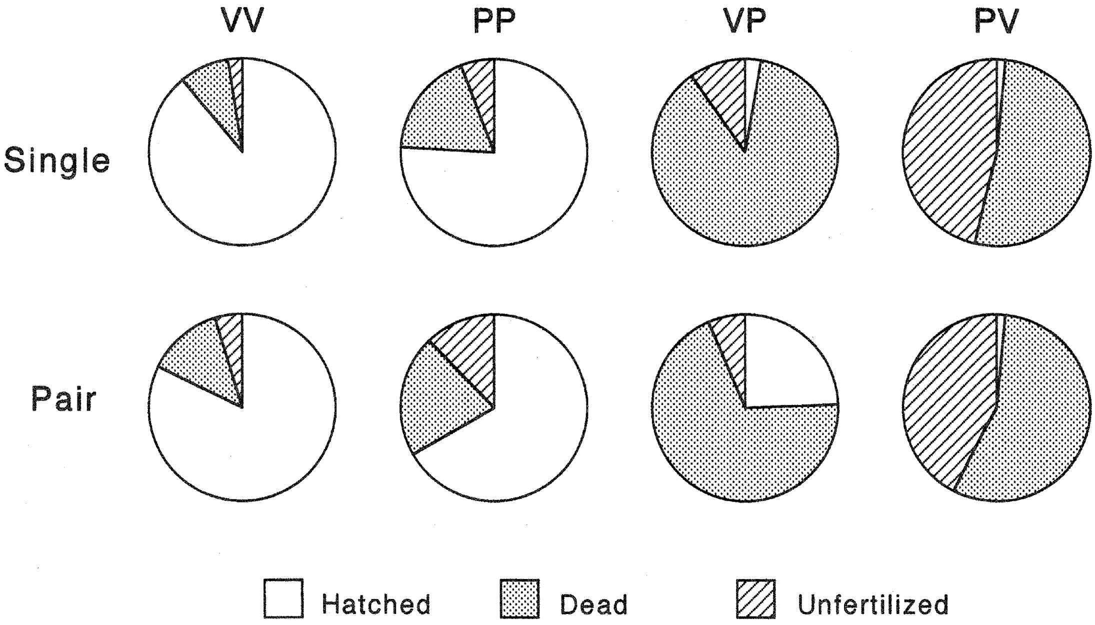

Fig. 2. The proportion of eggs that hatched, died during embryonic stages, or remained unfertilized in four combinations of crosses between *E. vigintioctomaculata* and *E. pustulosa* (based on Katakura and Sobu, 1986). Combinations of mating: VV, *vigintioctomaculata* females and *vigintioctomaculata* males; PP, *pustulosa* females and *pustulosa* males; VP, *vigintioctomaculata* females and *pustulosa* males; PV, *pustulosa* females and *vigintioctomaculata* males. Single: females were kept separate and alone after one mating. Pair: females remained paired with males, permitting repeated matings.

tion (Katakura, 1986a; Katakura and Sobu, 1986). It is obvious that the spermatozoa kept in the sperm reservoir of heterospecific females retain fertility to some extent. However, if they are physiologically or genetically anomalous due to the incompatibility with the genital tract of the females, this may result in the death of embryos. Thirdly, the lethality in hybrid embryos may be attributable to a discordance between the rate of cell division and that of chromosome replication during early embryogenesis (Tsurusaki *et al.*, 1993). This idea is derived from the fact that there is a quantitative difference in karyotypes between groups A and B (Tsurusaki *et al.*, 1993). The group B karyotype is characterized by a larger amount of heterochromatic segments on short arms of certain chromosomes than the group A karyotype.

Meanwhile, there is no effective isolating mechanism that acts after F_1 hybrids hatch. The F_1 larvae grow normally to become fully viable and fertile adults under laboratory conditions (Katakura and Nakano, 1979). Furthermore, the hatching rates of eggs laid by back crossings or crossings between F_1 hybrids are on the average higher than those by the interspecific matings (Nakano, 1981). Thus, reproductive isolation between *E. vigintioctomaculata* and *E. pustulosa* is attained by a combination of several kinds of barriers acting before hatching.

This pattern seems to be true for reproductive isolation between group A and group B in general. Except for "Western Tokyo form *Epilachna*" (=*E. yasutomii*), a pest of potato plants, host plants of the members of group B are different from those of group A. Furthermore, the hatching rates of eggs produced by crossings between groups A and B are consistently low (Table 4; Nakano, 1987; Tomioka, 1988; Katakura, unpubl.).

Putative hybrids between the two groups, identified on a morphological basis, are occasionally documented in natural conditions where the partial overlap of food plants violates ecological and phenological isolations (Nakano, 1987; Katakura, 1988). Furthermore, a contact zone is known in the Kanto district, middle Honshu, where *E. vigintioctomaculata* meets the "Western Tokyo form". The contact zone is narrow and hybrids are very rare, although the occurrence of F_1 and later filial hybrids was ascertained by analyzing allozymes (MDH and Malic enzymes) (Nakano, 1987; Takita and Katakura, unpubl.; Fig. 3). This fact indicates that host specificity is not a crucial mating barrier between group A and group B, and that the postmating barriers are more responsible for maintaining the morphological and biological integrity of the two groups in sympatry. This suggests an allopatric origin of the two groups, since postmating barriers are supposed to evolve as byproducts of genetic differentiation during isolation by extrinsic barriers (Coyne, 1992).

Conspecific sperm precedence and reproductive isolation

One noteworthy aspect derived from the study of reproductive isolation between the two groups of the *Epilachna vigintioctomaculata* complex is the role of conspecific sperm precedence as a reproductive barrier.

As already shown, the hatching rates of eggs produced by the interspecific matings of *E. vigintioctomaculata* and *E. pustulosa* are extremely low (Fig. 2). However, a low hatching

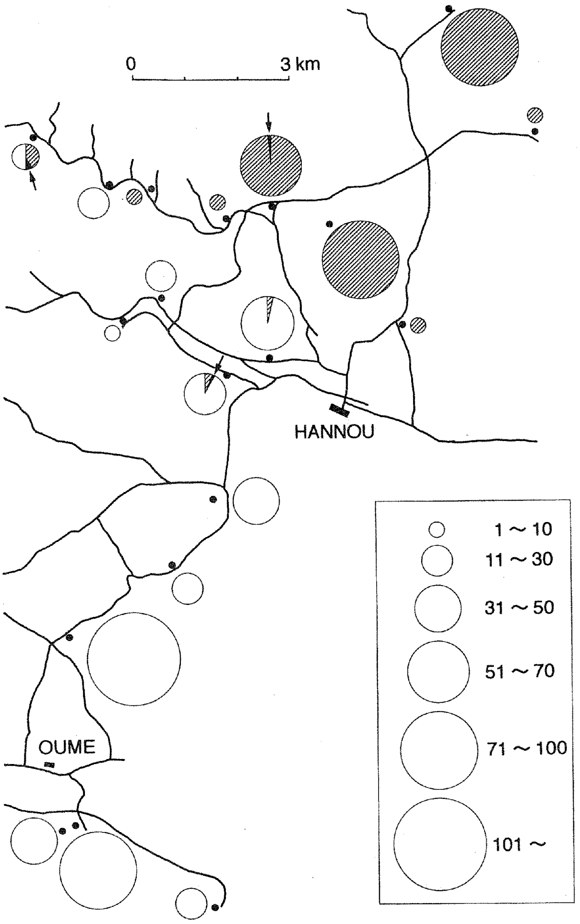

Fig. 3. The contact zone of *E. vigintioctomaculata* (hatched) and "Western Tokyo form *Epilachna*" (open) in the western part of the Kanto district, central Honshu (drawn based on unpublished data by H. Takita and Katakura). Occurrence of hybrids, confirmed by allozyme analyses, is designated by arrows (three populations).

rate due to an interspecific mating rose close to that of a conspecific mating immediately after a subsequent mating with a conspecific, and a high hatching rate by a preceding conspecific mating did not significantly drop after a succeeding interspecific mating (Table 5; Nakano, 1985). Moreover, almost all the progeny obtained from these doubly mated females were fathered by conspecific males (Table 6; Nakano, 1985). A subsequent study also confirmed very strong conspecific sperm precedence in this species pair (Katakura, 1986b).

Under such conditions, the number of offspring of interspecifically mated females must be greatly affected by their mating experience before and after interspecific matings, and females would suffer little reduction of the number of offspring from interspecific matings when they mate at least once with conspecifics before oviposition (Nakano, 1985). Indeed, the hatchability of eggs laid by females which had mated with heterospecifics in the field was not always low, and most of the offspring showed the mother's phenotypes (Nakano, 1987). Thus, available evidence strongly suggests that conspecific sperm precedence functions as a post insemination barrier between the two groups of the *E. vigintioctomaculata* complex.

Furthermore, conspecific sperm precedence may prevent reinforcement of premating isolation (Nakano, 1985, 1987; Katakura, 1986b). Theoretically, premating reproductive isolation can evolve at the zone of the contact of two incipient species at the expense of less fitted hybrid progeny (Dobzhansky, 1940). However, how common the reinforcement is in nature is still controversial (Butlin, 1987, 1989; Coyne and Orr, 1989; Howard, 1993). Studies of *Epilachna* beetles suggest that individuals may not suffer a reduction of fitness from interspecific matings owing to the strong conspecific sperm precedence (Nakano, 1985; Katakura, 1986b). This means that under certain conditions, selection for the reinforcement of premating isolation may be more relaxed than expected from the unfitness of hybrids.

Apparently, however, the effect of conspecific sperm precedence as a reproductive barrier is restrictive. Strong conspecific sperm precedence would serve most effective as a reproductive barrier when two species are equally abundant and females mate often (Gregory and Howard, 1994; Katakura, 1986b). Under such circumstances, a female is likely to mate at least once with a conspecific and therefore her eggs will show a normal level of hatchability. The female would suffer little reduction of fitness from interspecific mating. On the other hand, as the abundance of a species decreases, so too does the chance of a female of a species encountering and mating with a conspecific. The degree of conspecific sperm precedence itself also restricts its effectiveness as a reproductive barrier (Katakura, 1986b).

Thus, the general significance of conspecific sperm precedence in reproductive isolation is not yet fully understood. However, accumulating evidence suggests that reproductive

Table 5. Average hatching rates of eggs laid by doubly-mated females of *E. vigintioctomaculata* and *E. pustulosa* before and after the second mating (M2). Each female mates successively with a conspecific male (C) and a heterospecific male (H) (Nakano, 1985).

Order of matings	Species of female	N	Percentage hatch		Difference*
			before M2	after M2	
C - H	*vigintioctomaculata*	5	38.7	37.2	NS
	pustulosa	4	34.8	22.1	NS
H - C	*vigintioctomaculata*	7	8.9	33.7	+
	pustulosa	7	0.1	32.4	+

* NS, $p>0.05$; +, $p<0.05$ (t-test for paired comparison).

Table 6. Frequency of hybrids produced by doubly mated females of *E. vigintioctomaculata* and *E. pustulosa*. Each female mated successively with a conspecific male (C) and a heterospecific male (H) (Nakano, 1985).

Order of matings	Species of females	N*	Number of offspring		Frequency of hybrids (%)
			Non-hybrids	Hybrids	
C - H	*vigintioctomaculata*	5	306	2	0.7
	pustulosa	3	106	0	0.0
H - C	*vigintioctomaculata*	6	173	2	1.2
	pustulosa	6	192	1	0.5

*Number of females from which offspring are obtained.

Fig. 4. *Epilachna niponica* (left) and *E. yasutomii* (right) from Ohnuma, southern Hokkaido (same scale). Both individuals are female. Note the difference in body size and elytral shape.

Table 7. Effects of various factors (isolating mechanisms) on the reproductive isolation between *E. niponica* (En) and *E. yasutomii* (Ey)

Factor	Effect	Notes
Premating		
Ecological isolation	Very strong	Difference in host plants and in habitats of the host plants.
Seasonal isolation	None	Active periods overlap broadly.
Sexual (behavioral) isolation	None	Frequent interspecific matings under laboratory conditions; no tendency to choose conspecific mates.
Mechanical isolation	None	No significant difference in genitalic structure; sperm transfer between En and Ey normal.
Postmating (prezygotic)		
Gametic isolation	None	No reduction in the number of heterospecific spermatozoa kept by females; hatching rates of hybrid eggs normal.
Postmating (postzygotic)		
Hybrid inviability	None	Hybrid larvae are as viable as normal En and Ey larvae.
Hybrid sterility	None	Hybrids are as fertile as En and Ey.
Hybrid ecological unfitness	?	See discussion in text.

isolation at the gametic level may be a common phenomenon in closely related animal taxa (Howard and Gregory, 1993; Gregory and Howard, 1994; Albuquerque *et al.*, 1996).

Reproductive isolation between *E. niponica* and *E. yasutomii*

Among the three species of group B, *E. yasutomii* and *E. niponica* are sympatric from the southern part of Hokkaido to middle Honshu, occurring on different plants and having different morphology (Fig. 1, Table 1). *Epilachna niponica* is consistently and considerably larger (Fig. 4) and occurs on thistle, whereas *E. yasutomii* is smaller and occurs on blue cohosh and some subsidiary host plants. Furthermore, there are distinct differences in the elytral shape between the two sympatric species (Katakura, 1981a), and the differences are not attributable to allometry due to size difference.

Reproductive isolation between these two species has been most intensively studied using populations from Ohnuma, southern Hokkaido (Katakura *et al.*, 1981, 1989; Katakura and Hosogai, 1994, 1997). Unlike *E. vigintioctomaculata* and *E. pustulosa* mentioned above, *E. niponica* and *E. yasutomii* in

Ohnuma are isolated from each other by a strong premating barrier, their fidelity to different host plants (Table 7).

In Ohnuma, *E. niponica* occurs on thistle (most cases *Cirsium alpicola*) growing in forest-edge habitats and around marshes, whereas *E. yasutomii* occurs on blue cohosh on shaded forest floors. *Epilachna yasutomii* is also found feeding on *S. bryoniaefolius* in forest-edge habitats in the late summer to fall when blue cohosh withers. Coexistence of the two species on the same host species has not been documented. When given choices under laboratory conditions, adult beetles of either species prefer their own host plant to the host plant of other species (Table 8; Katakura *et al.*, 1989; Katakura and Hosogai, 1997). Furthermore, larvae are difficult to grow on the host plant of the other species (Fig. 5; Katakura *et al.*, 1989; Katakura and Hosogai, 1994). Since *Epilachna* beetles usually mate on the host plants, the observed strict host specificity must function as a very strong premating barrier for gene exchange between *E. niponica* and *E. yasutomii*.

On the other hand, no evidence for seasonal isolation, sexual isolation, gametic isolation, or reduced hybrid viability or fertility is known (Katakura *et al.*, 1981, 1989). Currently available data suggest that the active periods of adults of the two species overlap from early May through early September at Ohnuma. Furthermore, in spite of their difference in body size, *E. niponica* and *E. yasutomii* readily mate with each other under laboratory conditions without showing a particular preference for conspecifics, and produce normally viable and fertile hybrid offspring (Katakura *et al.*, 1981). The F_1 hybrid larvae can grow on both thistle and blue cohosh, and the F_1 adults easily accept both plants (Katakura and Hosogai, 1994,

Table 8. Food preference of newly emerged adults of *Epilachna niponica*, *E. yasutomii* and their F_1 hybrids for three plant species: blue cohosh (BC, host plant of *E. yasutomii*), thistle (TH, host plant of *E. niponica*) and *Solanum japonense* (NS, a plant preferred by both species in the laboratory)

Beetle species	N	Percentage of individuals that accepted during Period I			Percentage of individuals that accepted during Period I + II		
		thistle	blue cohosh	*S. japonense*	thistle	blue cohosh	*S. japonense*
E. niponica	29	86.2	0.0	100.0	100.0	31.0	100.0
NY hybrids	30	33.3	96.7	100.0	100.0	100.0	100.0
YN hybrids	51	51.0	88.2	94.1	100.0	100.0	100.0
E. yasutomii	36	0.0	97.2	91.7	33.3	100.0	100.0

Results for beetles reared on these three plants are pooled. Each beetle was allowed to choose between the three plants during the first three days (Period I) and then forced to accept the plant(s) that was not eaten during Period I for up to six days (Period II). 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. (Modified from Katakura and Hosogai, 1997).

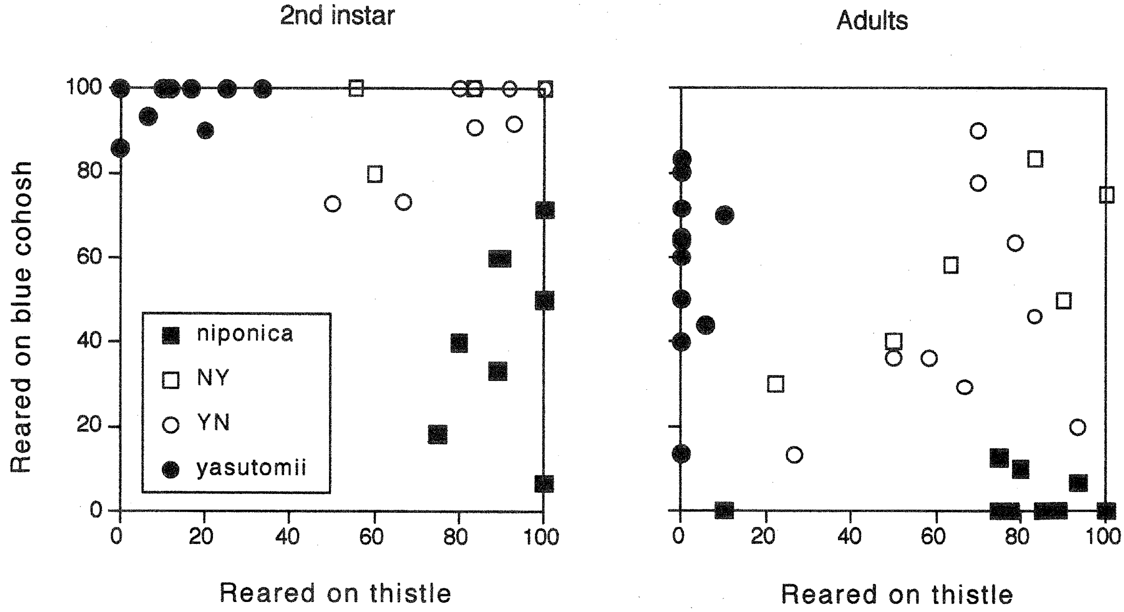

Fig. 5. Rate of survival for *Epilachna niponica*, *E. yasutomii* and their hybrids on thistle (host of *E. niponica*) and blue cohosh (host of *E. yasutomii*) (based on Katakura and Hosogai, 1994). Percentage survival to the second instar (left) and that to adulthood (right) are given separately. Offspring of a single female were divided into two groups and reared on different plants. Each dot represents the survival rates of offspring from a single female.

1997).

At a glance, such reproductive isolation which is totally dependent on host fidelity appears to be very fragile. Although habitats of thistle and blue cohosh are different, the two host plant species occasionally grow side by side in ecotones. Given such circumstances, there is always the potential for interspecific mating, should errors in host choice occur. As there are no effective postmating barriers, occasional hybridization, if it occurs, should obscure the distinctness between the species by subsequent gene flow. However, this is apparently not the case. The two species are sympatric and keep their morphological and biological distinctness from southern Hokkaido to central Honshu (Katakura, 1981a). One explanation for this is that host selection is sufficiently strict and completely prevents hybridization of the two species under natural conditions. Another explanation is that hybrids are produced but, because they are considerably less adaptive than their parents in some ecological properties, for example, in the phenological correspondence with host plants, resistance to natural enemies, or competitive ability to exploit host plants, they are selected against.

Thus, although host preference seems to act as a very strong and probably unique premating barrier to gene flow between *E. niponica* and *E. yasutomii*, it is still uncertain whether occasional hybridization occurs or not under natural conditions. For a thorough understanding of reproductive isolation between the two species, we need to know much more about the 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.

The speciation process of *E. niponica* and *E. yasutomii* is also not clear. Some authors believe that speciation in phytophagous insects can occur without geographic isolation (Bush, 1969, 1975, 1994; Tauber and Tauber, 1989), and the members of the *E. vigintioctomaculata* complex are among such candidates for sympatric speciation (Diehl and Bush, 1984; Bush and Howard, 1986; Tauber and Tauber, 1989). The above-mentioned mode of reproductive isolation between *E. niponica* and *E. yasutomii* satisfies certain conditions necessary for some models of sympatric speciation via a host shift (e.g. Bush, 1974; Diehl and Bush, 1989). Again, however, the critical point, a reduction of hybrid fitness has not yet been demonstrated. Furthermore, little is known about the genetic basis of host specificity in these beetles (but see Ueno *et al*., 1997).

The relationship among the three species of group B

As mentioned above, *E. niponica* and *E. yasutomii* are distinct biological entities that warrant treatment as different biological species, even though their reproductive isolation is attained by host fidelity alone. However, the relationship between either of the two species and *E. pustulosa*, their northern counterpart, is enigmatic. *Epilachna pustulosa* is distributed in Hokkaido except for the southern and eastern parts, and is allopatric with both *E. niponica* and *E. yasutomii* (Fig. 1). In the Oshima Peninsula, southern Hokkaido, *E. pustulosa* and the two southern species (*E. niponica* and *E. yasutomii*) are separated by a narrow zone not occupied by any members of group B (Fig. 1). Body size of *E. pustulosa* is, on average, intermediate between *E. niponica* and *E. yasutomii* (Katakura, 1981a). Furthermore, the elytral shape cannot be used as a reliable character that separates the northerly distributed *E. pustulosa* from southerly distributed *E. niponica* and *E. yasutomii*, since there is considerable geographic variation in the elytral shape of both *E. niponica* and *E. pustulosa* (Fig. 1).

Crossing experiments revealed that *E. pustulosa* and the other two species produced fully viable and fertile hybrids (Katakura *et al*., 1981). Moreover, there seems to be no effective premating barrier between *E. niponica* and *E. pustulosa* or between *E. yasutomii* and *E. pustulosa*, because the so-called Sapporo form of *E. pustulosa* (form P-III in Fig. 1) distributed just north of *E. niponica* and *E. yasutomii* occurs on both thistle and blue cohosh (Katakura, 1976; Kimura and Katakura, 1986). Indeed, host preference and the performance of the Sapporo form of *E. pustulosa* under laboratory conditions are very similar to those of the F_1 hybrids of *E. niponica* and *E. yasutomii* (Table 9; Fig. 6; Katakura, unpubl.). These lines of evidence indicate that *E. pustulosa* cannot be discriminated from either *E. niponica* or *E. yasutomii*. *Epilachna pustulosa* is "conspecific" with both *E. niponica* and *E. yasutomii*, nevertheless the latter two are "heterospecific". In other words, group B behaves as one species in the north but as two separate species in the south. The situation is further

Table 9. Food preference of posthibernating adults of *E. pustulosa* (Sapporo form) collected on thistle and blue cohosh

Collected on	N	Percentage of individuals that accepted during Period I		Percentage of individuals that accepted during Period I + II	
		thistle	blue cohosh	thistle	blue cohosh
thistle	28	75.0	96.4	92.9	100.0
blue cohosh	30*	50.0	100.0	89.3	100.0

The beetles were allowed to choose between the two plants during the first five days (Period I) and then forced to accept the less preferred plant during the next five days (Period II). N = The number of individuals examined. (Katakura, unpubl. data).
*N = 28 in Period II.

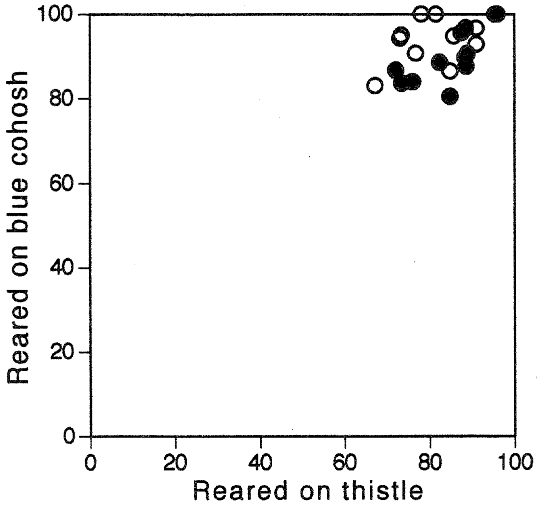

Fig. 6. Rate of survival for *Epilachna pustulosa* (Sapporo form) on thistle and blue cohosh. Offspring of females collected on thistle and blue cohosh are shown by open and solid circles, respectively. For further explanation, see Fig. 5 (Katakura, unpubl. data).

complicated by the fact that the preference of *E. pustulosa* for blue cohosh decreases in the northern parts of the distribution range (Hoshikawa, 1984). The recognition of the three species in group B is therefore operational, since such a complex situation cannot be adequately treated by conventional taxonomy.

At the present, it is not obvious how such a situation evolved. Group B might be in the process of gradual splitting, assuming that the Sapporo form of *E. pustulosa* is an ongoing sympatrically speciating population. Alternatively, it might be in the process of convergence owing to hybridization at the zone of secondary overlap following allopatric divergence. On the other hand, there might be a very complex situation involving partial divergence and partial hybridization.

Conclusion

The mode of reproductive isolation is diverse among the members of the *Epilachna vigintioctomaculata* complex as summarized above. Several factors function as barriers to gene flow between group A and group B. Postmating-prezygotic barriers, thus far little studied in animals, are considered to be of primary importance to maintaining the distinctness of members of the two groups in sympatry.

On the other hand, *E. niponica* and *E. yasutomii*, two sympatric species of group B, are reproductively isolated from each other by a single premating barrier, fidelity to different host plants. Their relationship is, however, complicated by the occurrence of an allopatric third "species", *E. pustulosa*, that shows an intermediate host specificity and presumably lacks any potential barriers to gene exchange with either *E. niponica* or *E. yasutomii*.

These findings suggest that the mode of speciation may also be diverse among the *E. vigintioctomaculata* complex. For groups A and B, an allopatric origin is more likely, but for the members within group B, an origin without geographic isolation is also plausible.

Attempts are being made to clarify the phylogenetic relationships of the members of the *E. vigintioctomaculata* complex (N. Kobayashi *et al.*, unpubl.), extent of gene flow between sympatric taxa, genetic basis of host specificity (Ueno *et al.*, 1997; Ueno, unpubl.), and extrinsic and intrinsic factors responsible for the process of host selection in natural conditions (Koizumi *et al.*, 1997, unpubl.; Fujiyama and Katakura, 1997; Fujiyama, unpubl.). These studies will greatly enrich our knowledge of the evolutionary history of the *E. vigintioctomaculata* complex, and will contribute to better understanding of animal species and their origin.

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