Res Popul Ecol (1999) 41:107–119

SPECIAL FEATURE

Naota Ohsaki · Yoshibumi Sato

The role of parasitoids in evolution of habitat and larval food plant preference by three *Pieris* butterflies

Received: December 3, 1998 / Accepted: January 20, 1999

Abstract This article attempts to explain that parasitoids provide the evolutionary pressure responsible for relationships between habitat use and larval food plant use in herbivorous insects. Three species of butterflies of the genus *Pieris*, *P. rapae*, *P. melete*, and *P. napi* use different sets of cruciferous plants. They prefer different habitats composed of similar sets of cruciferous plants. In our study, *P. rapae* used temporary habitats with ephemeral plants, *P. melete* used permanent habitat with persistent plants, although they also used temporary habitats, and *P. napi* used only permanent habitat. The choice experiment in the field cages indicated that each of the three butterfly species avoided oviposition on plants usually unused in its own habitat, but accepted the unused plants which grew outside its own habitat. Their habitat use and plant use were not explained by intrinsic plant quality examined in terms of larval performance. *Pieris* larvae collected from persistent plants or more long lasting habitats were more heavily parasitized by two specialist parasitoids, the braconid wasp *Cotesia glomerata* and the tachinid fly *Epicampocera succincta*. The results suggest that *Pieris* habitat and larval food plant use patterns can be explained by two principles. The evolution of habitat preference may have been driven by various factors including escape from parasitism. Once habitat preference has evolved, selection favors the evolution of larval food plant preferences by discriminating against unsuitable plants, including those which are associated with high parasitism pressures.

Key words Alternative factors · Habitat permanence · Herbivore · Plant quality · Three trophic levels

N. Ohsaki (✉)
Laboratory of Insect Ecology, Graduate School of Agriculture,
Kyoto University, Kyoto 606-8502, Japan
Tel. +81-75-753-6134; Fax +81-75-753-6474
e-mail: ohsaki@kais.kyoto-u.ac.jp

Y. Sato
Kyoto College of Medical Technology, Sonobe, Funai-gun, Kyoto
622-0041, Japan

Introduction

It has been debated whether the behavioral evolution of herbivorous insects is part of a coevolutionary process involving just two trophic levels (plants and herbivores) or whether three trophic levels (plants, herbivores, and the predators and parasites of the herbivores) are involved (e.g., Price et al. 1980; Bernays and Graham 1988). Resolution of this problem is important for understanding behavioral evolution in herbivorous insects and therefore the evolution of their community structure. However, there is still little empirical evidence showing the relative importance of these two types of interactions in nature. In particular, few convincing examples exist of the relative importance of variation among food plant species in predator or parasite load, and its interaction with variability in the intrinsic quality of those food plants, in the evolution of food plant preference. In this study, we provide an example of this phenomenon.

When attempting to understand the factors that influence the evolution of food plant use by herbivorous insects, a convenient hypothesis is that preference rankings primarily reflect relative differences in the intrinsic quality of food plants such as chemistry, nutritional content, and architecture, as reflected in juvenile survival and growth rates (Thompson 1988). Under this hypothesis, herbivorous insects would be expected to prefer plants of high intrinsic quality. If they prefer unexpected plants, there would be other factors influencing the evolution of food plant preference. Failure of herbivorous insects to use the food plants of highest intrinsic quality has been documented previously (Chew 1975; Wiklund 1975; Smiley 1978; Courtney 1981; Jermy and Szentesi 1983; Kearney 1983; Rausher 1983; Rowell 1985; Roininen and Tahvanainen 1989; Ohsaki and Sato 1994). However, only in a few of these studies have other possible selective factors influencing food plant use also been examined.

Thompson (1988) outlined four types of alternative selective factors that should be considered when intrinsic quality of food plants fails to explain patterns of food plant use by herbivorous insects: (1) relative abundance and intrinsic quality of food plants vary spatiotemporally; (2) herbivores have been exposed to different food plants for different periods of time; (3) intrinsically inferior food plants may be favored if intrinsically superior food plants are too small to support the complete development of larvae; and (4) intrinsically inferior food plants may be favored if herbivores are more susceptible to predation or parasitism on intrinsically superior food plants. Also (5), interspecific competition seems another possible alternative factor.

In the field, the set of food plants used by a herbivorous insect is largely determined by the behavior of the egg-laying female. For a female to lay eggs on an appropriate food plant, she must pass through at least two successive steps: (1) location of the larval food plant habitat, and (2) location of the larval food plant itself within the habitat. Populations or species subjected to the same evolutionary pressures may evolve different behavioral mechanisms for ensuring that the same set of food plants is used. In particular, different species may, in theory, evolve to avoid using unsuitable food plants, on which larvae have poor survival as a result of various factors such as low intrinsic plant quality or high natural enemy load, by terminating search at either of these two steps. Under this view, the primary selection pressure responsible for habitat restriction is avoidance of unsuitable food plants. An alternative view is that selective pressure unrelated to food plant suitability (e.g., thermal requirements, absence of nearby nectar sources, etc.) is primarily responsible for restricted habitat use. In this case, selection for avoidance of unsuitable food plants is manifested primarily by behavior to refrain from ovipositing on such food plants that occur in the habitat used. In this study we attempt to determine which of these alternatives better explains habitat and host plant use in three species of *Pieris* butterflies by examining, at least to some extent, the foregoing five alternative selective factors.

Three *Pieris* species – *P. rapae* Boisduval, *P. melete* Menetries, and *P. napi* japonica Shirozu – use different sets of cruciferous larval food plants (Ohsaki 1979). In addition, the three species prefer different habitats, which are composed of similar sets of crucifers (Ohsaki 1982). *P. rapae* uses ephemeral food plants such as cultivated crops and weeds in a sunny field. This species has six or seven generations in our census area, and its females are effective colonizers (Ohsaki 1980). *P. melete* and *P. napi* use persistent plants that tend to be longer lasting and occur in shaded locations. They have four generations, and their females tend to be sedentary (Ohsaki 1980). However, *P. melete* often lays eggs on all crucifers used by *P. rapae* in the mountainous area. *P. melete*, therefore, is a polyphagous species and *P. rapae* is a relatively oligophagous species. On the other hand, usually a local *P. napi* population uses only one *Arabis* species because species in the genus *Arabis* have a disjunct distribution.

In Japan, *Pieris* larvae are attacked by two specialist parasitoids, a braconid wasp, *Cotesia glomerata* L. (formerly referred to as *Apanteles glomeratus*) (Nagashima 1933; Clausen 1940; Feltwell 1981), and a tachinid fly, *Epicampocera succincta* Meigen (Yasumatsu and Watanabe 1964; Iwao et al. 1989; Iwao and Ohsaki 1996). A *C. glomerata* female lays about 30 eggs in larvae of each of first three instars of the three *Pieris* species, but *C. glomerata* larvae egress from fifth-instar larvae of only *P. rapae* and *P. napi*. The eggs laid in the body of *P. melete* are encapsulated (Sato 1976). *E. succincta* usually places a first-instar offspring in either the fourth or fifth instars of the three *Pieris* species larvae, and one fly larva egresses from each host pupa of the three *Pieris* species. When *C. glomerata* and *E. succincta* parasitize the same larvae of *P. rapae* and *P. napi*, only parasitism by *C. glomerata* succeeds (Iwao and Ohsaki, unpublished data). When both parasitoids use the same host larvae of *P. melete*, only *E. succincta* parasitizes successfully because *C. glomerata* eggs are encapsulated (Iwao and Ohsaki, unpublished data).

In this study we report the results of a survey of comparative habitat use and larval food plant use by the three *Pieris* species, as well as of oviposition choice test in each habitat. Furthermore, we assess the permanence of each habitat by temporal distribution patterns of larval populations of *Pieris* species. We also document the relative intrinsic quality of each food plant to the three *Pieris* species by measuring their larval performance. We assess relative acceptability of food plants to ovipositing females in the field cage and document their parasitism rates by the wasp *C. glomerata* and the fly *E. succincta* on each food plant of the pierid larvae in each habitat. Finally, we examine the relationship between habitat permanence and parasitoid pressure. The primary purpose of this study is to use this information to argue that the differences in habitat use and food plant use among the three *Pieris* species are most easily interpreted as resulting from a trade-off between parasitoid avoidance and the intrinsic quality of plants.

Materials and methods

Study area

The study area (about 12 km from south to north and 6 km from east to west) was in the northern part of Kyoto City (35.0° N, 135.8° E), consisting of lowland and mountainous areas. Here, nine sites (A–I) were chosen for the census (Fig. 1). Sites A through C were sunny agricultural fields in the lowland area and were more than 1 km from the forest edge, and Sites D through I were located in the mountainous area. Each site was isolated by paddy fields, dense human habitation, or forest composed primarily of Japanese cedar (*Cryptomeria japonica* D. Don.). The area of sites varied, but mean area was about 1 ha.

Within all the sites, cultivated crucifers such as white cabbage, (*Brassica oleracea* L.), Chinese cabbage (*B. pekinensis* Rupr.), and Japanese radish (*Raphanus sativus* L.) were periodically planted in the fields of scattered small farms and around houses. A wild crucifer, *Rorippa indica* (L.) Hieron, which has six or seven generations per year, occurred around gardens and cultivated fields and along

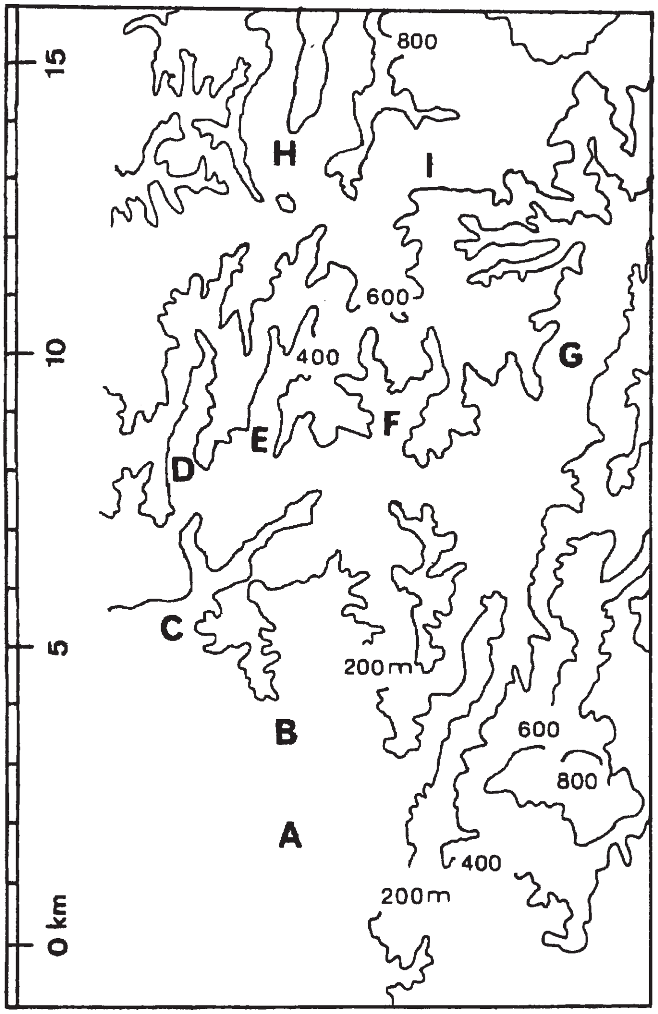

Fig. 1. Map of the study area. There are nine census sites (A–I): *A* through *C*, sunny agricultural fields in the lowland area; *D* through *I*, in the mountainous area

roads. In some sites in the mountainous area, a semiwild plant, *Wasabia japonica* (Miq.) Matsumura that is cultivated locally and a wild species, *Cardamine appendiculata* Fr. et Sav., grew along the edges of small streams along the edge of forest from early spring to late autumn. In addition, a wild species, *Arabis gemmifera* Makino, grew concealed with other weeds and bushes under Japanese cedar throughout the year.

The mountainous site was basically composed of four subsites: the sunny agricultural field, the field edge, the edge of the forest, and the bare floor of the forest interior. The sunny agricultural fields in the mountainous area were more than 5 m from the forest edge. The field edge was located within 5 m of the edge of the forest, which frequently became shaded during the day. The forest edge was a strip about 2 m wide with weeds and bushes under Japanese cedar. The bare floor of the forest interior was more than 1 m from the forest edge. Therefore, the study area was categorized into five habitat types: the sunny agricultural field in the lowland area (a), the sunny agricultural field in the mountainous area (b), the field edge (c), the forest edge (d), and the bare floor of the forest interior (e) (Fig. 2). We used all sites for the census of *Pieris* larvae to assess parasitism rate, but used some sites for other censuses and experiments.

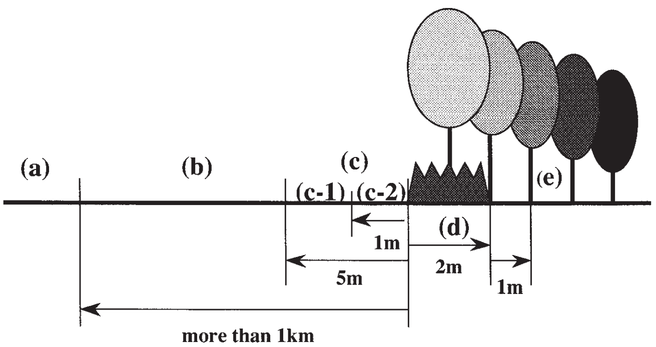

Fig. 2. Schematic illustration of habitat types. The study area was categorized into five habitat types, (*a*)–(*e*): the sunny agricultural field in the lowland area (*a*); and the mountainous area composed of four habitat types, a sunny agricultural field (*b*), the field edge (*c*), the forest edge (*d*), and the bare floor of the forest interior (*e*). In addition, the field edge is divided into two habitats, the edge of the field (*c-1*) and the field–forest boundary (*c-2*)

Distribution of naturally occurring eggs in habitats and on larval food plants

To determine which habitat types are used naturally, and which of the seven cruciferous species are used as larval food plants by each of the *Pieris* species, we chose six of nine sites: A, B, C, D, F, and H. Sites A, B, and C were sunny agricultural fields in the lowland area. Because Sites D, F, and H, which were in the mountainous area, had relatively complete sets of all habitat types other than (a), they were chosen for this census. The numbers of eggs of each *Pieris* species were censused for each cruciferous plant species at each habitat type of each site chosen in early July 1989. Each cultivated species was assessed by 50 haphazardly chosen shoots at each habitat type of each site. For each wild crucifer species, 250 shoots were chosen at each habitat type of each site, because its biomass was very small. However, as *R. indica* was very rare in habitat type (c), we chose all its shoots found there. Eggs of the three *Pieris* butterflies were easily discriminated by their differences in size, shape, and color (Shirozu and Hara 1971).

Experimental determination of habitat use of the three *Pieris* butterflies

A census of naturally occurring eggs can give a misleading picture of the habitats searched (utilized) by ovipositing females if some habitats that are searched contain few individual plants of acceptable cruciferous species. Consequently, the purpose of this experiment was to determine whether few eggs are found in particular habitats because butterflies avoid those habitats or simply because there are no acceptable food plants within those habitats. By placing

R. sativus, a cruciferous species acceptable to all three butterfly species, in each habitat, failure to find eggs of a given species indicated avoidance of that habitat.

Site F was chosen for the experiment because it had a complete set of all habitat types other than (a) in the mountain area. In each of its habitat types (c) and (d), 140 potted Japanese radish (*R. sativus*) plants were placed and left for 3 days in early July 1989. In addition, 70 potted *R. sativus* were also placed at the habitat type (e). Normally, there are no cultivated crucifers at these habitat types at any time during the year. At the habitat type (c), 35 potted radish plants were placed in each of the two rows at the field edge between 1 and 5 m from the edge of forest (c-1), and at the field–forest boundary within 1 m exterior from the edge of forest (c-2), with plants spaced about 10 cm apart within rows. At the habitat type (d), two double rows of 35 plants were placed about 30 m apart, and the plants were naturally concealed by the native vegetation. Therefore, twice as many potted plants were placed here as in other habitat types. At habitat type (e), two rows of 35 potted radish plants were placed. The Japanese radish plants for this experiment were grown from seed in 8-cm-diameter pots. Because there were many *R. sativus* planted at habitat types (a) and (b) at census periods, we did not place any *R. sativus* there.

The permanence of habitats of the three *Pieris* butterflies

As an index of the permanence of each habitat type, we estimated the continuous generation numbers of the three *Pieris* species after their spring appearance in each habitat type. We collected any fourth- and fifth-instar *Pieris* larvae found on all crucifer species of each habitat type of six sites chosen for the census of distribution of naturally occurring eggs. Census was conducted at least twice a month from April to November in 1984. This census was a part of a census for determining the parasitism rates of parasitoids described later in detail.

Intrinsic quality of potential larval food plants

To assess the intrinsic quality of the seven crucifers for *Pieris* larvae, we measured survival rates and developmental times of larvae and pupal masses on each crucifer from June to July in 1979. All the *Pieris* larvae were obtained from female butterflies captured in the northern part of Kyoto and induced to oviposit in cages of *B. pekinensis* (*P. rapae* and *P. melete*) or on *Arabis hirsuta* (*P. napi*). Within 24 h of hatching, first-instar larvae were placed individually on fresh leaves of each plant with a fine hair pencil. The leaves were kept moist by placing them with wet absorbent cotton in 200-ml plastic cups. The larvae were reared at a constant temperature of 25°C with a 16:8 day:night light cycle. Leaves were replaced daily. The sample size for each food plant species × *Pieris* species combination was 30. These experimental conditions are within the normal range experienced by larvae of all three *Pieris* species in the field (Ohsaki 1982).

All cultivated crucifers used were planted in a field at Kyoto University, fertilized with oil cakes and dolomite, and collected daily. *W. japonica*, *R. indica*, *C. appendiculata*, and *A. gemmifera* were collected in the mountainous area in northern Kyoto every 3–4 days. These wild plants packed in plastic bags and stored in a dark chamber at 5°C.

Development time was measured as the time between hatching and pupation; individual pupal masses were measured between 24 and 36 h after pupation.

Relative acceptabilities of the potential larval food plants to ovipositing females

To quantify the relative acceptabilities of the seven cruciferous species to the three pierids, preference tests were conducted in ourtdoor cages ($1.8 \times 1.8 \times 1.8\,\mathrm{m}^3$) in a field at Kyoto University in early July 1985. One plant pot of each of the seven cruciferous species was placed in a circle in the cage. In a given trial, a female of each pierid species was introduced into the cage for 24 h and the eggs laid on the plants were subsequently counted. The experiment consisted of a total of 8 trials for *P. rapae* and *P. napi* and 11 trials for *P. melete*, each with a different set of females and with a different set of pot plants arranged in a different, haphazardly chosen order.

All butterfly females were collected from Site F 1 day before their experiments, and were kept in plastic bags and placed in the laboratory. All cultivated crucifers for this experiment were grown from seed in 12-cm-diameter pots. All wild plants were transplanted from Site F to 12-cm-diameter pots about 2 months before this experiment. No plant was reused in any experiment.

The cover effect on ovipositing females caused by concealing larval food plants with other vegetation

Almost all *P. napi* eggs were laid on *Arabis* plants, which were usually concealed with other vegetation and were difficult to find. Therefore, we attempted to determine whether, for *P. napi*, locating weedy habitats is a necessary prerequisite to initiating search for larval food plants, i.e., whether weeds are releasers for alighting on and “tasting” individual food plants, or whether this behavior can occur in open (non-weedy) habitats. In early July 1985, We constructed both a concealed habitat and an open habitat in an outdoor cage identical to that described for the previous experiment, except that this cage had been overgrown with weeds. The west half of the cage floor was kept unweeded to provide a concealed habitat, while the weeds growing in the east half of the cage were removed to provide open habitat. Four potted *Arabis gemmifera* plants were placed on the floor of each half of the cage. Four *P. napi* females were introduced into the cage and left for 24 h, after which the eggs laid on the host plants were counted. The plants used in this experiment were collected from Site F and transplanted into 8-cm-diameter pots 2 months before the experiment. *P. napi* butterflies were also collected from Site F 1 day before the experiment.

Parasitism rates of the three *Pieris* larvae in the field

To assess the parasitism rates by *C. glomerata* and *E. succincta* for each habitat type and the seven cruciferous plants, all crucifer species at all the habitat types of all the census sites were examined at least twice a month from April to November in 1984, and fifth-instar larvae found were collected. When there were many larvae, we sampled haphazardly, but when there were few larvae we attempted to find them all.

To determine parasitism rates, collected *Pieris* larvae were reared individually in 200-ml plastic cups on *B. oleracea* or *R. sativus* in the laboratory until either parasitoids or adult butterflies emerged. When *E. succincta* larvae emerged from *Pieris* pupae, we assumed these were parasitized by only *E. succincta*. When *C. glomerata* larvae egressed from *Pieris* larvae, the *Pieris* larvae were dissected to determine if *E. succincta* larvae were present, because when *C. glomerata* and *E. succincta* parasitize in the same host larvae of *P. rapae* and *P. napi*, only parasitism by *C. glomerata* succeeds and the body of the *E. succincta* larva is left in the body of its host larva of *Pieris*. The body of the *E. succincta* larva was easily identified from the shape of its hard mandible with a binocular microscope.

Habitat permanence and seasonality affecting parasitism rates

To assess the parasitism rates affected by habitat permanence and seasonality, the rates of parasitism of *P. rapae* by *C. glomerata* in habitat types (a) and (b) were compared. We based four habitat categories on habitat permanence and seasonality: spring and other seasons for newly established habitats, and after early summer and autumn for long-lasting habitats.

Results

Distribution of naturally occurring eggs in habitats and on larval food plants

The three pierid species all differed in the distribution of naturally laid eggs among habitats. *P. rapae* eggs were found almost exclusively in the sunny agricultural field in the lowland habitat type (a) and the sunny agricultural field in the mountainous area (b), where the three cultivated food plant species and *R. indica* were all used. By contrast, very few *P. rapae* eggs were found in the field edge habitat (c); these eggs were all laid on *R. indica* (Table 1).

Pieris melete eggs were distributed differently among habitats. They were found in the sunny agricultural field in the mountainous area (b) and in the field edge habitat (c). All host species in these habitats were utilized, although some more heavily than others (e.g., two cultivated crucifers, *B. oleracea* and *B. pekinensis*, as well as *W. japonica*., were used less frequently than the other species present). Interestingly, *P. melete* eggs were absent from the sunny agricultural field in the lowland area (a), even though the same food plant species grew there as were present in the sunny agricultural field in the mountainous area (b) (Table 1).

Pieris napi exhibited the narrowest natural distribution, most eggs being confined to the forest edge habitat (d), although a few eggs were laid on *R. indica* in the field edge habitat (c). This species also exhibited a narrower food plant range than the other two species; most eggs were laid on a single food plant species, *A. gemmifera* (see Table 1). *R. indica* was the only plant species on which all three *Pieris* butterflies laid eggs in habitat type (c), but this plant species was very rare there.

Experimental determination of habitat use of the three *Pieris* butterflies

The purpose of this experiment was to determine whether the absence of eggs resulted from lack of searching for plants or the lack of acceptable plants; this was tested by providing the mutually acceptable *R. sativus*. At the sunny field in both the lowland area (a) and the mountainous area (b), *R. sativus* was already present. The sunny agricultural field in the lowland (a) was used by only *P. rapae*, and that in the mountainous area (b) was used by both *P. rapae* and *P. melete* (Table 1). The field edge habitat (c) was searched by females of all three species (Table 2). This area was divided into two strips: the field edge (c-1) and the field forest boundary (c-2). Of these two strips, *P. rapae* apparently searched both with equal intensity, while *P. melete* concentrated more on the edge of field (1–5 m), while *P. napi* concentrated primarily on the field–forest boundary (within 1 m). At the forest edge habitat (d), where the potted radish plants were naturally concealed by weeds in conditions similar to those under which *A. gemmifera* grew, only *P. napi* laid eggs (Table 2), suggesting that this was the only pierid to search in this habitat. The *Pieris* butterflies, even *P. napi*, never laid eggs on potted *R. sativus* on the bare floor in the forest interior (e). *P. napi* constrained their range of habitats at the forest edge (d) and field–forest boundary (c-2). These results indicate that, for each butterfly species, butterflies did not search for food plants in the habitats from which naturally laid eggs were absent (Fisher's PLSD, $P < 0.05$).

Permanence of habitats of the three *Pieris* larvae

As an index of the permanence of each habitat type, we estimated the continuous generation numbers of the three *Pieris* larvae after spring appearance in each habitat type. The potentially annual generation number of *P. rapae* was estimated to be about six or seven in the lowland and six in the upland, while those of *P. melete* and *P. napi* was estimated to be four in the mountainous area (Ohsaki and Sato 1990). Of the three census programs of habitat type (a), one larval population of *P. rapae* at Site A and two of those at Sites B and C underwent crashes within one and two generations, respectively, after their spring appearance because

Table 1. Numbers of eggs (mean ± SD) of the three *Pieris* species on each shoot of cruciferous plant species

Pieris species	Habitat type	Plant species	Sample size	A	B	C	D	F	H
P. rapae	a	*Brassica oleracea*	50	1.94 ± 1.53	3.19 ± 3.12	2.92 ± 3.37	–	–	–
		B. pekinensis	50	0.06 ± 0.24	0.06 ± 0.31	0.60 ± 1.19	–	–	–
		Raphanus sativus	50	0.08 ± 0.27	0.31 ± 0.55	0.76 ± 1.49	–	–	–
		Rorippa indica	250	0.04 ± 0.20	0.12 ± 0.39	0.90 ± 1.71	–	–	–
	b	*B. oleracea*	50	–	–	–	2.52 ± 1.92	15.60 ± 8.96	0.60 ± 0.75
		B. pekinensis	50	–	–	–	0.70 ± 0.79	0.10 ± 0.30	No plant
		R. sativus	50	–	–	–	0.35 ± 0.67	0.82 ± 1.10	0.75 ± 1.08
		R. indica	250	–	–	–	0.26 ± 0.44	0.35 ± 0.62	0.23 ± 0.77
	c	*Cardamine appendiculata*	250	–	–	–	No plant	0	0
		Wasabia japonica	250	–	–	–	No plant	0	0
		R. indica		–	–	–	0.26 ± 0.45	0.43 ± 0.50	0.21 ± 0.42
			(*n*)				<23>	<30>	<38>
	d	*Arabis gemmifera*	250	–	–	–	0	0	0
	e	No crucifer							
P. melete	a	*B. oleracea*	50	0	0	0	–	–	–
		B. pekinensis	50	0	0	0	–	–	–
		R. sativus	50	0	0	0	–	–	–
		R. indica	250	0	0	0	–	–	–
	b	*B. oleracea*	50	–	–	–	0.08 ± 0.27	0.83 ± 1.44	0.32 ± 0.63
		B. pekinensis	50	–	–	–	0.33 ± 0.67	0.34 ± 0.77	No plant
		R. sativus	50	–	–	–	0.40 ± 0.60	1.73 ± 2.82	1.73 ± 2.82
		R. indica	250	–	–	–	1.10 ± 0.99	1.18 ± 2.01	0.10 ± 0.31
	c	*C. appendiculata*	250	–	–	–	No plant	0.78 ± 1.36	0.32 ± 0.65
		W. japonica	250	–	–	–	No plant	0.11 ± 0.39	0.22 ± 0.77
		R. indica		–	–	–	0.90 ± 1.02	0.56 ± 0.99	3.57 ± 4.27
			(*n*)				<23>	<30>	<38>
	d	*A. gemmifera*	250	–	–	–	0	0	0
	e	No crucifer							
P. napi	a	*B. oleracea*	50	0	0	0	–	–	–
		B. pekinensis	50	0	0	0	–	–	–
		R. sativus	50	0	0	0	–	–	–
		R. indica	250	0	0	0	–	–	–
	b	*B. oleracea*	50	–	–	–	0	0	0
		B. pekinensis	50	–	–	–	0	0	No plant
		R. sativus	50	–	–	–	0	0	0
		R. indica	250	–	–	–	0	0	0
	c	*C. appendiculata*	250	–	–	–	No plant	0	0
		W. japonica	250	–	–	–	No plant	0	0
		R. indica		–	–	–	0.17 ± 0.38	0.10 ± 0.30	0.34 ± 0.15
			(*n*)				<23>	<30>	<38>
	d	*A. gemmifera*	250	–	–	–	0.25 ± 0.58	0.20 ± 0.40	0.28 ± 0.70
	e	No crucifer							

Habitat type: a, sunny agricultural fields in the lowland area; b, sunny agricultural fields in the mountainous area; c, the field edge; d, the forest edge; and e, the bare floor of the forest interior

The plant size and amount of plant searched were very different among plant species

Table 2. Oviposition of three *Pieris* butterflies on potted *Raphanus sativus* placed at each habitat type where *R. sativus* usually is not planted under natural conditions

Habitat type	No. potted *R. sativus*	No. of eggs per shoot (mean ± SE)		
		P. rapae	*P. melete*	*P. napi*
c-1	70	0.10^{a} ± 0.04	0.99^{a} ± 0.11	0.14^{a} ± 0.05
c-2	70	0.13^{a} ± 0.05	0.43^{b} ± 0.07	0.57^{b} ± 0.08
d	140	0^{b}	0^{c}	0.04^{ac} ± 0.02
e	70	0^{b}	0^{c}	0^{c}

Site F: (c-1), the field edge between 1 m and 5 m from the edge of forest; (c-2), the field edge within 1 m exterior from the edge of forest; (d), the forest edge; (e), the bare floor of the forest interior. Data were analyzed by Fisher's PLSD for each species. Within each column, numbers followed by different letters are significantly different ($P < 0.05$)

Table 3. Three measures of success for *Pieris* larvae fed on leaves of different food plants

Species	*n*	Larval survival	Males			Females		
			n	Duration of larval stage (d)	Pupal mass (mg)	*n*	Duration of larval stage (d)	Pupal mass (mg)
P. rapae								
B. oleracea	30	100	18	11.8^{b} ± 0.2	197.6^{a} ± 5.2	12	11.5^{abc} ± 0.3	193.0^{a} ± 6.1
B. pekinensis	30	100	18	10.4^{a} ± 0.1	189.4^{a} ± 6.0	12	10.3^{ab} ± 0.1	168.1^{b} ± 3.2
R. sativus	30	100	16	10.7^{a} ± 0.2	189.2^{a} ± 4.1	14	10.6^{ab} ± 0.1	163.0^{b} ± 4.7
R. indica	30	100	15	10.8^{a} ± 0.2	200.0^{a} ± 4.7	15	11.3^{abc} ± 0.2	179.2^{c} ± 3.3
C. appendiculata	30	83.3	12	12.9^{c} ± 0.3	170.9^{b} ± 5.0	12	12.5^{bc} ± 0.2	165.2^{b} ± 3.2
W. japonica	30	46.7	8	15.0^{d} ± 0.3	129.5^{c} ± 4.6	6	15.7^{d} ± 0.6	122.2^{d} ± 3.0
A. gemmifera	30	73.3	9	23.3^{e} ± 1.2	112.2^{d} ± 6.0	13	22.5^{e} ± 1.6	97.9^{e} ± 4.2
P. melete								
B. oleracea	30	100	9	13.4^{a} ± 0.2	267.8^{ab} ± 8.3	21	13.6^{a} ± 0.2	206.3^{b} ± 3.5
B. pekinensis	30	100	20	12.0^{b} ± 0.2	266.9^{ab} ± 6.9	10	12.2^{b} ± 0.4	233.2^{a} ± 6.9
R. sativus	30	100	15	12.9^{a} ± 0.3	249.5^{ac} ± 6.2	15	13.6^{a} ± 0.2	224.2^{a} ± 9.1
R. indica	30	100	11	13.3^{a} ± 0.1	236.0^{c} ± 3.5	19	13.9^{a} ± 0.3	236.7^{a} ± 3.4
C. appendiculata	30	100	18	12.1^{b} ± 0.2	251.7^{abc} ± 8.3	12	18.1^{b} ± 0.3	233.3^{a} ± 4.8
W. japonica	30	100	21	15.2^{d} ± 0.2	204.6^{d} ± 3.6	9	16.0^{c} ± 0.3	184.1^{c} ± 5.2
A. gemmifera	30	96.7	7	19.0^{e} ± 0.5	207.9^{e} ± 7.6	22	18.1^{d} ± 0.3	195.3^{bc} ± 4.6
P. napi								
B. oleracea	30	100	15	13.1^{bc} ± 0.3	180.1^{adef} ± 4.3	15	14.2^{bc} ± 0.3	178.7^{bcd} ± 5.2
B. pekinensis	30	100	15	12.6^{abc} ± 0.2	196.7^{b} ± 5.2	15	13.3^{ab} ± 0.1	218.5^{a} ± 5.4
R. sativus	30	100	15	12.4^{ab} ± 0.2	196.7^{cd} ± 5.2	15	14.0^{bc} ± 0.2	209.4^{a} ± 3.6
R. indica	30	100	15	12.8^{abc} ± 0.1	232.4^{cd} ± 2.9	15	13.1^{a} ± 0.2	217.6^{a} ± 2.9
C. appendiculata	30	100	14	13.0^{abc} ± 0.2	196.1^{cd} ± 5.6	16	12.9^{a} ± 0.2	183.8^{bc} ± 3.5
W. japonica	30	100	15	14.9^{d} ± 0.4	175.5^{ef} ± 5.1	15	14.5^{bc} ± 0.3	176.6^{bcd} ± 4.8
A. gemmifera	30	100	22	14.4^{d} ± 0.1	191.5^{cde} ± 5.6	8	14.0^{bc} ± 0.3	164.8^{cd} ± 5.1

d, days

Data are mean ± SE. Duration of the larval stage and pupal mass were analyzed by Bonferroni multiple range test for each sex separately. Within each column, numbers followed by different letters are significantly different ($P < 0.05$)

cultivated crucifers decreased markedly in abundance or disappeared. New cultivated fields were established in all three programs in the year, and larval populations of *P. rapae* were then also re-established. Thus, habitat type (a) was a temporary habitat.

Of the three census programs of habitat type (b), larval populations of *P. rapae* continued for two (Site F), three (Site D), and six (Site H) generations, respectively, after their spring appearance, and those of *P. melete* continued for two, two, and four, respectively. Thus, habitat type (b) was more long-lasting but also temporary. In two of the three census programs of habitat type (c), larval populations of *P. melete*, which was the representative species in this habitat type, continued for four generations (Sites F and H), although in that of habitat (d) we could not find any mature larvae. In all of the three census programs of habitat type (d), larval populations of *P. napi* continued for four generations. Therefore, both habitat types (c) and (d) were permanent habitats. In summary, *P. rapae* used temporary habitats, *P. melete* used permanent habitats, although they also used temporary habitats, and *P. napi* used only permanent habitats.

Intrinsic quality of potential larval food plants

Three cultivated crucifers (*B. oleracea*, *B. pekinensis*, and *R. sativus*) and *R. indica* were relatively intrinsically superior plants for the three *Pieris* butterflies, as assessed by larval performance (Table 3). In particular, *B. oleracea* was the most intrinsically superior plant for *P. rapae*, but was not as good for *P. napi*. *C. appendiculata* was also intrinsically superior. On the other hand, *W. japonica* and *A. gemmifera* were intrinsically inferior. In particular, *A. gemmifera* was the least suitable even for *P. napi*, which used it almost exclusively in the field (Table 1).

Relative acceptabilities of the potential larval food plants to ovipositing females

The three pierid species also exhibited different oviposition preferences for the seven potential food plant species in the choice experiment, indicating evolutionary divergence in behavior associated with oviposition. *P. rapae* exhibited a strong preference for *B. oleracea*. Nevertheless, the three food plant species (*C. appendiculata*, *W. japonica*, and *A. gemmifera*) growing naturally in permanent habitats, habitat types (c) and (e), were oviposited on much less frequently than the cultivated crucifers and *R. indica*, but not significantly (e.g., compared with *R. indica*, which alone was used by *P. rapae* as the wild plant, *P* values of the Bonferroni multiple range test of the other food plants were as follow: *B. oleracea*, <0.0001; *B. pekinensis*, 0.73; *R. sativus*, 0.37; *C. appendiculata*, 0.18; *W. japonica*, 0.11; *A.*

gemmifera, 0.11) (Fig. 3). *P. melete*, in contrast, was more of a generalist with respect to oviposition preferences.

All food plant species except *A. gemmifera* were oviposited (e.g., comparing with *C. appendiculata*, which is considered to be the original food plant of *P. melete*, *P* values of the Bonferroni multiple range test of the other plants were as follows: *B. oleracea*, 0.03; *B. pekinensis*, 0.04; *R. sativus*, 0.95; *R. indica*, 0.92; *W. japonica*, 0.21; *A. gemmifera*, 0.07) (Fig. 3). Finally, *P. napi* was intermediate between the other two species in breadth of food plant choice, preferring the native *A. gemmifera* and *R. indica* and the cultivated species *B. pekinensis* and *R. sativus* over the other species (e.g., comparing with *A. gemmifera*, which is the original food plant of *P. napi*, *P* values of the Bonferroni multiple range test of the other plants were as follows: *B. oleracea*, 0.03; *B. pekinensis*, 0.32; *R. sativus*, 0.15; *R. indica*, 0.28; *C. appendiculata*, 0.002; *W. japonica*, 0.002) (Fig. 3).

These results permit two conclusions. First, each of the three butterfly species avoids ovipositing on the species that grow in the habitats normally used but upon which they do not oviposit in nature. For example, *P. napi* discriminated against *C. appendiculata* and *W. japonica* in the choice experiment, and these are precisely the plants that are not used by these species in permanent habitat (c) (Table 1). Second, for *P. napi*, two naturally unused species, *B. pekinensis* and *R. sativus*, are acceptable when encountered. These species are apparently not used in nature because they are restricted to habitats not used by *P. napi*.

The cover effect on ovipositing females caused by concealing larval food plants with other vegetation

Pieris napi females laid eggs on all potted plants of *A. gemmifera* on the bare floor (13.50 ± 2.10, mean ± SE), while no eggs were laid on any plant concealed by weeds. It thus appears that weedy vegetation is not a required releaser for alighting and oviposition in this species (U-test of Mann–Whitney; $P = 0.014$). This result is consistent with results of the previous experiment in which *P. napi* laid more eggs on *R. sativus* when the plants were not concealed in the field edge habitat (c) than on plants that were concealed in the forest edge habitat (d).

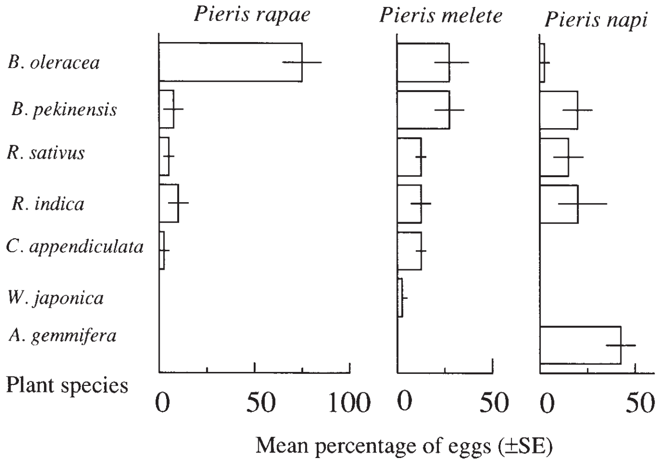

Fig. 3. Mean percentage of eggs (±SE) laid on each shoot of seven cruciferous host species by three *Pieris* species in outdoor cages

The parasitism rates of the three *Pieris* larvae in the field

The parasitism rates by both *C. glomerata* and *E. succincta* differed among habitats as well as among the three pierid species. In the lowland temporary habitats (a), only *P. rapae* larvae lived and were parasitized by *C. glomerata* on each food plant (16%–29%). Its parasitism rate did not significantly differ among the food plant species ($\chi^2 = 3.57$, df = 3, $P = 0.31$). The parasitism rates by *E. succincta* there were very low (0%–2%) (Fig. 4).

In the mountainous temporary habitats (b), where *P. rapae* and *P. melete* lived, the parasitism rates of *P. rapae* by *C. glomerata* (61%–86%) were higher than those in the lowland temporary habitats ($\chi^2 = 146.14$, df = 1, $P < 0.0001$). However, its parasitism rates significantly differed among the food plant species ($\chi^2 = 8.29$, df = 3, $P = 0.04$) (Fig. 4). *P. melete* larvae were not parasitized by *C. glomerata*. Although *C. glomerata* frequently attacks *P. melete* in the field (13%–48%, Sato and Ohsaki 1987), *P. melete* larvae were not successfully parasitized by *C. glomerata* (Fig. 4) because wasp eggs were killed in the bodies of *P. melete* larvae by encapsulation by host hemocytes (Sato 1976). On the other hand, parasitism rates of *P. rapae* by *E. succincta*, which did not significantly differ among the plant species ($\chi^2 = 3.81$, df = 3, $P = 0.28$), were lower than those by *C. glomerata* ($\chi^2 = 147.94$, df = 1, $P < 0.00001$) (Fig. 4). In addition, the rates of *P. rapae* by *E. succincta* were not different from the parasitism rates of *P. melete* by *E. succincta* on each plant species ($\chi^2 = 0.35$, df = 1, df = 1, $P = 0.55$) (Fig. 4).

In the mountainous permanent habitats (c), where all three pierid species lived, only *R. indica* was used by all of them, although this plant species was very rare there (Table 1). We could not collect any of their fifth-instar larvae. The other food plants, *C. appendiculata* and *W. japonica*, in habitat type (c) were used by only *P. melete*. We collected its fifth-instar larvae from *C. appendiculata*, and its parasitism rate by *E. succincta* was significantly higher than that on other food plants of habitat type (c) (73%, $\chi^2 = 24.03$, df = 1, $P < 0.0001$) (Fig. 4). *P. melete* larvae on *C. appendiculata* were also parasitized by *C. glomerata*, in spite of the fact that the result was unsuccessful parasitism (55.3%; Sato and Ohsaki 1987).

In the forest edge habitats, habitat type (d), which are normally concealed by other weeds (Sato and Ohsaki 1987), almost all *P. napi* larvae were collected from *A. gemmifera*. On such plants, *P. napi* larvae were rarely parasitized by either *C. glomerata* (3%) or *E. succincta* (2%) (Fig. 4). However, some larvae were collected from *A. gemmifera* from which weeds had either been irregularly removed or which had grown unexpectedly exposed on a stone wall along a road. The total parasitism rate on these exposed plants by the two parasitoids combined was much higher (83%) ($\chi^2 = 53.19$, df = 1, $P < 0.0001$). These results suggest

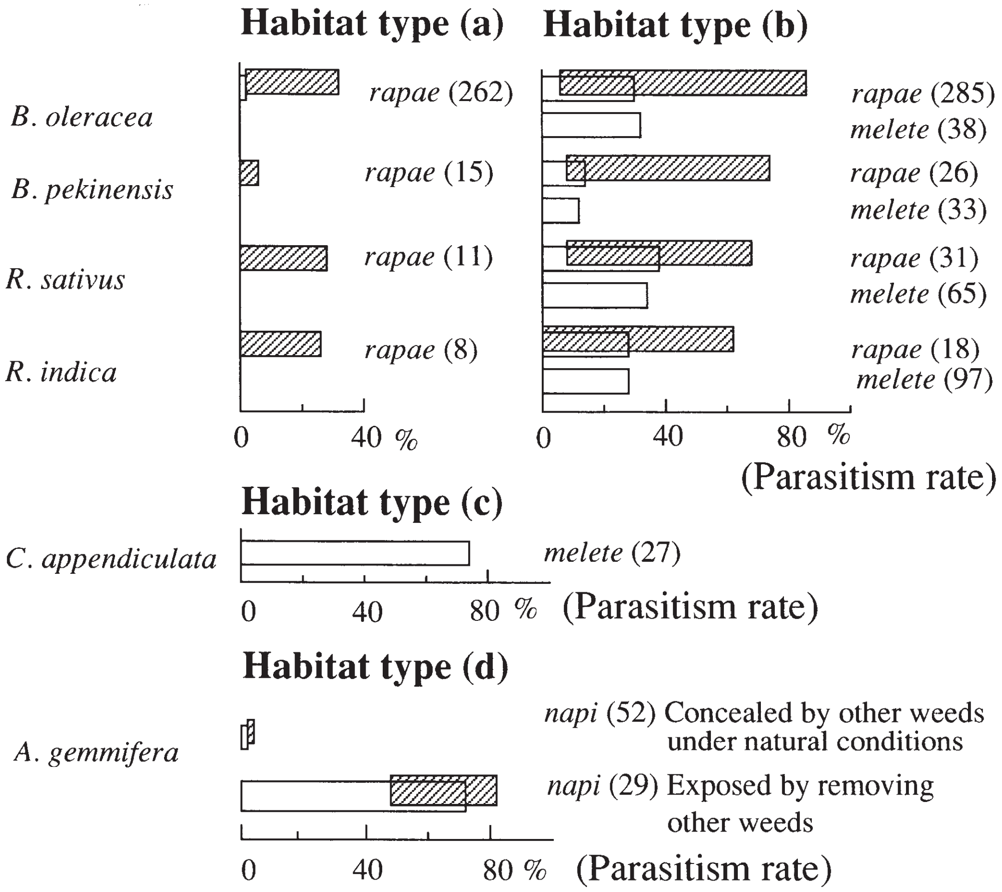

Fig. 4. Parasitism rates of three *Pieris* species by *Cotesia glomerata* (*shaded area*) and *Epicampocera succincta* (*white area*) on each cruciferous plant at each habitat type. Larvae parasitized by both species are indicated by *shaded* and *white areas*. Numbers in parentheses are sample size of fifth-instar larvae

that vegetation concealing *A. gemmifera* protects *P. napi* larvae from parasitoid attack, and that if *P. napi* had laid eggs on crucifers other than *A. gemmifera* under natural conditions, its larvae would have been highly parasitized.

Habitat permanence and seasonality affecting parasitism rates

The parasitism rates of *P. rapae* by *C. glomerata* and *E. succincta* differed between the lowland temporary habitat (a) and the mountainous temporary one (b) (Fig. 4). This difference was likely to be caused by the difference in frequency of habitat structure in which *P. rapae* larvae were collected (Fig. 5). We made four habitat categories based on habitat permanence and seasonality. When new fields of cultivated crucifers were established partway through the year, larval populations of *P. rapae* also increased. The parasitism rates of *P. rapae* collected from such newly established habitats were low (10%–23%). On the other hand, the rates of parasitism of *P. rapae* collected from long-lasting habitats, except the final generations in late autumn, were high (82%–87%). The parasitism rates of the first generations in early spring and the final generations of the year (about six generations per year; Ohsaki and Sato 1990) were low (20%–26%). These annual patterns of change in parasitism rate did not differ much between the lowland and mountainous areas [regression between the parasitism rates of the montainous area (y) and those of the lowland (x) to assess the relationship between parasitism rates and habitat categories is as follows: $y = 1.026x + 2.751$, $r^2 = 0.999$, $P = 0.003$] (Fig. 6). However, frequencies of larvae collected from each category differed between lowland and mountainous area ($\chi^2 = 172.71$, df = 3, $P < 0.0001$) (Fig. 5). Therefore, higher parasitism rates in the mountainous area were the result of the nature of the habitat, which was frequently more long-lasting than those of lowland areas.

Thus, in the temporary habitats, parasitoid pressure is low, but even in the temporary habitats, parasitism rates of continuous populations increase steadily. In the permanent habitats, the parasitoid pressure is potentially high.

Discussion

The results of this study suggest that the habitat- and larval food plant use patterns of the three *Pieris* species studied can be explained by two principles:

1. The evolution of habitat preference is driven by a variety of factors, including strategies for escaping parasitism.
2. Once habitat preference has evolved, selection favors evolution of larval food plant preferences for suitable plants and discrimination against unsuitable plants on which larvae have poor survival because of high rates of parasitoid attack or low intrinsic plant quality.

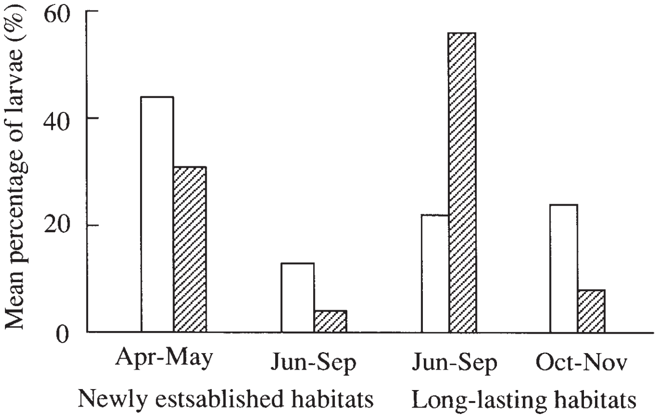

Fig. 5. The number of fourth- and fifth-instar larvae of *Pieris rapae* collected from two types of habitats: newly established (*left*) and long-lasting (*right*) habitats in both lowland and mountainous areas. *White areas* indicate *P. rapae* collected from the sunny agricultural field in the lowland area; *shaded areas* indicate those in the mountainous area

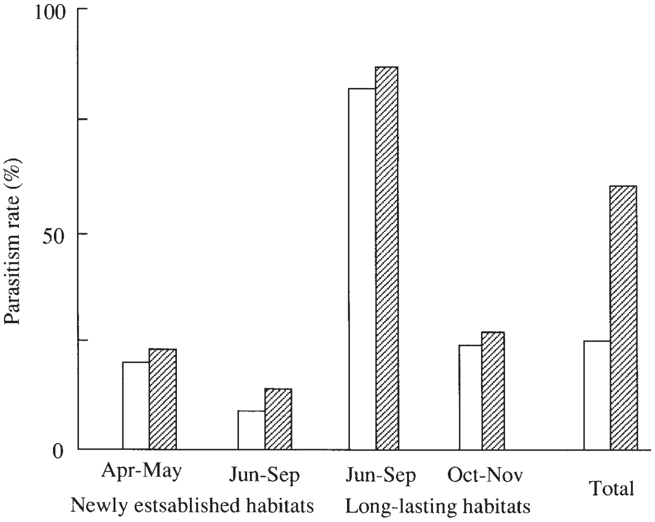

Fig. 6. Parasitism rates of *P. rapae* by *C. glomerata* collected from two types of habitats: newly established and long-lasting habitats in both the lowland and mountainous area. The larvae collected are fourth and fifth instars. *White areas*, *P. rapae* collected from the sunny agricultural field in the lowland area; *shaded areas*, those from the mountainous area

In the following paragraphs, we discuss the evidence supporting each of these principles.

Evolution of habitat preference

The potential habitat available to these *Pieris* species constitutes the entire range of environments in which their potential food plants (crucifers) grow. These environments can be divided into two categories: (1) temporary habitats in which potential food plants, largely crops, are ephemeral, and (2) permanent habitats in which food plants are more persistent.

All three *Pieris* utilize only a portion of the entire range of habitats available, as demonstrated both by censuses of egg distributions on naturally growing plants and by assessment of egg distributions on plants known to be acceptable to ovipositing females that were planted throughout the range of habitats (Table 1). Moreover, food plants on which *P. melete* and *P. napi* oviposited in the habitats normally utilized are also available in other habitats but are not used (*B. oleracea*, *B. pekinensis*, *R. sativus*, and *R. indica* for *P. melete*; *R. indica* for *P. napi*), suggesting that these habitats are not searched by the females (see Table 2).

These observations indicate that habitat use by the three butterfly species is restricted to a subset of the range of habitats that contain acceptable food plants. In turn, this observation suggests that availability of acceptable food plants does not always govern evolution of habitat preference. Rather, limits on the range of acceptable habitats seems to be governed by other types of selection pressures. The results of this study suggest what some of these selection pressures may be. Parasitism rates of the three *Pieris* larvae, for example, are on average much higher in permanent habitats than in temporary habitats (Figs. 4 and 6), presumably because in permanent habitats parasitoid populations can build up to high equilibrium levels; that is, habitat permanence and parasitoid load are correlated. Differential parasite pressure and differential avoidance mechanisms by the three *Pieris* species against the parasitoids can thus explain why they use only a particular portion of the entire range of habitat available.

For *P. napi*, the reason for confinement to forest edge habitat (d) is clear and may be evidence for the proposition that habitat preference is governed by factors other than intrinsic quality of host plants. Almost all *P. napi* larvae were collected from *A. gemmifera* (Fig. 4), the most intrinsically inferior plants (see Table 3; Yano and Ohsaki 1993), in forest edge habitat (d), but when *P. napi* larvae are accidentally collected from the cruciferous plants in other than forest edge habitat, they are heavily attacked and killed by *C. glomerata* (Sato and Ohsaki 1987; Ohsaki and Sato 1990, 1994) and *E. succincta* (Ohsaki and Sato 1994). By contrast, parasitism rates are minimal for larvae collected from *A. gemmifera* in forest edge habitat (d) (see Fig. 4). This difference in susceptibility in different habitats may result from differences in the degree to which the plants in the forest edge habitat (d) are concealed by surrounding vegetation (Fig. 4). *A. gemmifera* normally grows concealed below surrounding vegetation. When the surrounding vegetation was artificially removed, however, *P. napi* larvae were heavily parasitized (Fig. 4), indicating that naturally low parasitism rates in this habitat result from vegetation impeding the ability of parasitoids to locate larvae. These observations are thus consistent with the interpretation that *P. napi* has evolved to specialize on an intrinsically inferior food plant because of selection imposed by parasitoids and has been restricted in the use of permanent habitat.

Although the plant *R. indica* is a utilized host, like all other potential food plants except *A. gemmifera* its suitability for *P. napi* larval survival is very poor because of parasitism (Sato and Ohsaki 1987; Ohsaki and Sato 1990, 1994). Presumably, *R. indica*, an ephemeral plant normally growing in temporary habitats, is an unexpected plant at field edge habitats (c), because this plant species was very rare in this habitat type (Table 1). Therefore, restriction of habitat use to forest edges may be an evolved mechanism that facilitates restriction of oviposition to *A. gemmifera*, on which larvae escape parasitism (Fig. 4).

If *R. indica* is an unexpected plant in the field edge habitat (c), there are no food plants utilized by *P. napi* in this strip (c) next to the forest edge (d), although *P. napi* frequently lay eggs on experimentally placed potted *R. sativus* in the field edge habitat (c). Nevertheless the strip next to the forest edge is an important part of the habitat of *P. napi* because *Pieris* butterflies must keep their body temperatures at nearly 31°C for their normal behaviors. The thermal environment at the forest edge under Japanese cedar is less than 25°C; thus, *P. napi* must frequently fly to the strip next to the forest edge to bask in solar radiation to elevate their body temperature (Ohsaki 1986).

Habitat restriction in *P. melete* also seems to be unrelated to avoidance of poor intrinsic quality of host plants. This species is restricted to mountainous habitats, even though temporary lowland habitats with a similar set of host plants as those in temporary mountainous habitats are available (Table 1). Previous results suggest that this restriction is because temperatures in lowland areas exceed the thermal tolerance of this species (Ohsaki 1982; Nagasaka 1992).

In mountainous habitat, *P. melete* does not use forest edge habitats (d), although they use all other cruciferous plants in all other mountainous habitats such as habitat type (b) and (c). The most obvious difference in selection pressures acting on habitat use by the three *Pieris* butterfly species that we identified is their difference in susceptibility to *C. glomerata*. *P. melete* has evolved the ability to encapsulate eggs of this wasp and is therefore immune to its attack (Sato 1976). Hence, unlike *P. napi*, there is no reason for *P. melete* to use forest edge habitat (d) with only *A. gemmifera*, plants of intrinsically most inferior quality (Table 3).

However, *P. melete* suffers heavily from parasitism by *E. succincta*, in particular on *C. appendiculata* in habitat type (c) (Fig. 4). Habitat type (c) with *C. appendiculata* is likely to be the original habitat with original food plants used by *P. melete*, because this plant is the only native species in the diet of *P. melete* in our study area. Therefore, utilization of habitat (b) by *P. melete*, which is a new habitat for this species, may have been accelerated by parasitism by *E. succincta*. That is, parasitoid pressure may function as force polyphagy for *P. melete* but as monophagy for *P. napi*.

The habitat use patterns of *P. rapae* can also be interpreted as being influenced by parasitoids. *P. rapae* larvae collected from long-lasting habitats were more heavily parasitized than those from newly established habitats (Fig. 6). Newly emerged females of *P. rapae* always disperse from their natal habitats even if those habitats are suitable for their offspring (Ohsaki 1979, 1982). Therefore, these habitats may be used usually not by individuals born there but by recruits from other habitats. However, by exploiting newly opened habitats, *P. rapae* may colonize before parasitoids occur such that *P. rapae* larvae often will have passed through the first three instars most susceptible to parasitism before the parasite arrives. In addition, only *P. rapae* among the three *Pieris* species lays eggs in the sun, so their incubation time is reduced by about two-thirds compared to eggs laid in the shade (Ohsaki 1982). Consequently, this increases their possibility of escaping from *C. glomerata*. Therefore, *P. rapae* mainly use temporary habitats such as habitat type (a) and (b), which generally suffer lower parasitoid pressure (see Fig. 4).

Although *P. rapae* uses only temporary habitats, they may not discriminate against habitats with high parasitoid pressure. Those habitats are longer lasting, and often built adjacent to old habitats. In such case they suffer very heavy attack by the parasitoids. However, such long-lasting habitats are usually built in mountainous areas on a small scale. Most temporary habitats are built in lowland areas in large scale and are purely temporary habitats.

Evolution of food plant preference within utilized habitats

While restricted habitat use may not contribute to discrimination against food plants that do not occur in the utilized habitats, not all food plants available in the utilized habitat are used (Table 1, Fig. 3). Moreover, this study demonstrates that many of these available but nonutilized food plants are actively discriminated against by females searching for oviposition sites (Table 1, Fig. 3). For example, *P. napi* lays eggs on *R. indica* in field edge habitats (c), but not on *C. appendiculata* or *W. japonica*. As mentioned earlier, if *R. indica* is an unexpected plant there, choice tests (Fig. 3) indicate that active discrimination against these species explains this pattern. Similarly, in the field *P. rapae* lays most of its eggs on *B. oleracea*e, and the choice tests indicate this plant is preferred strongly to the others available in its normally utilized habitat (Fig. 3). Finally, *P. melete* exhibits similar preferences for *R. indica* and *C. appendiculata* over *W. japonica*, although in this case the trend in the preference tests was not statistically significant (Fig. 3).

This type of discrimination among available food plant species has been documented in many prior studies of butterfly preference (e.g., Singer 1971; Chew 1975; Rausher 1980). Such a pattern is normally attributed to differences in food plant suitability: preferences are expected to evolve for food plant species on which egg and larval survival is highest. This explanation will hold for the three *Pieris* species if we consider parasitoid pressure as a part of the unsuitability of food plants. Thus, for *P. napi*, which prefers *A. gemmifera* to *W. japonica* and *C. appendiculata* in its utilized habitat, survival would be much lower on the latter two plants because of attack by parasitoids. *P. melete* appears to have evolved to avoid *W. japonica* because of its intrinsically low quality as a substrate for growth (Table 3). Finally, although *W. japonica* and *C. appendiculata* both occur in habitats used by *P. rapae*, this butterfly discriminates strongly against them (Fig. 3). Larvae on these two species have poor survival because of high parasitoid attack (both plant species; Fig. 4) and low intrinsic host quality (*W. japonica*; Table 3).

Historical considerations

The foregoing considerations have ignored differences among the *Pieris* species in historical associations with host plants, which may also be expected to influence our interpretation of the evolutionary processes that have governed habitat and food plant preference. *P. napi* and *P. melete* are native to Japan, and have presumably been associated with native crucifers for long periods of time. Before extensive habitat disturbance by humans, it is likely that native species of crucifers (i.e., *C. appendiculata*, *W. japonica*, *A. gemmifera*) grew only in mountainous undisturbed habitats such as along forest edges bordering streams. Only in historical times have both mountainous and lowland disturbed habitats, and the crop species they contain, become available to *P. melete* and *P. napi*. *P. melete* has "capitalized" on this opportunity by expanding its range into mountainous disturbed habitats to use cultivated crucifers. As a result, *P. melete* suffers lower parasitoid pressure in mountainous disturbed habitats than in their original habitat (Fig. 4). These facts indicate that such expansion is possible, although of course we lack definitive evidence that such an expansion was the result of an evolutionary change in behavior. However, the similarity of assemblage of available food plants between mountainous and lowland temporary habitats, with rather more suitable plants in the lowland, suggests that failure of *P. melete* to expand into the lowlands is actively opposed by some sort of selection. As suggested here, one candidate for such selection is lack of tolerance to lowland thermal conditions. By contrast, *P. napi* has failed to expand its range into these newly available habitats (Table 1), despite the presence of intrinsic high-quality food plants (crops) (Table 3). This failure to expand its habitat range may reflect simple lack of genetic variability for habitat preference (i.e., a remnant of previous evolutionary history). However, it is also clear that when such variation does arise, it is likely to be selected against because parasitoid pressure on *P. napi* at such newly available habitat is intense.

Although for both of these species it is difficult to determine definitively whether natural selection opposes habitat expansion or whether lack of expansion is the result of recent contact with new habitats, in both species host discrimination among available food plants seems less likely to be explainable by such an historical factor. In particular, both species exhibit discrimination against native crucifer species that grow in presumably ancient natural habitats.

Pieris rapae, unlike the other two butterfly species, is a relatively recent arrival in Japan. Throughout much of the world, this species inhabits temporary habitats and utilizes cultivated as well as native crucifers. Its confinement largely to this type of habitat and these types of species in Japan may thus also be ascribed to recency of invasion and lack of sufficient time for genetic variation in behavior to arise that would allow habitat expansion into permanent habitats. However, it is also clear that when such variation does arise, it is likely to be selected against because parasitoid pressure on *P. rapae* is much more intense in permanent than in temporary habitats (Figs. 4 and 6). Thus, ultimately, habitat restriction to temporary habitats is likely to be maintained by natural selection.

The other alternative factors

Interspecific competition seems another possible alternative factor in habitat choice and food plant choice. However, we have not found any indication to show interspecific competition among the three *Pieris* species. Strong et al. (1984) reviewed studies reporting either the presence or absence of interspecific competition among herbivorous insects, concluding that interspecific competition among herbivorous insects may be relatively rare. Therefore, interspecific competition may not be the primary factor of habitat choice and food plant choice by the three *Pieris* species.

The other two alternative factors proposed by Thompson (1988) are (1) spatiotemporal variation of relative abundance and quality of food plants; and (2) use of intrinsically inferior food plants that are too small to support the complete development of larvae. It seems unlikely that these two factors contribute significantly to the observed differences in habitat use and food plant use of the three *Pieris* butterflies, because all larval food plants censused in this study were used by at least one of the three species of *Pieris*.

However, factor (1) may explain an interesting fact not shown in this study. Only *P. melete* laid eggs on *Wasabia japonica* in this study, in early July. Before and after this season, this plant is not used even by *P. melete* (Ohsaki and Sato 1994). Presumably, the intrinsic quality of this plant varies temporally.

Our work on *Pieris* suggests that the most possible factor affecting the evolution of their habitat- and food plant use patterns is escape from parasitism. We infer that specialist parasitoids may be one of the most important ecological factors affecting the evolution of habitat and food plant preferences in herbivorous insects. We expect that additional examples will be found as more plant–herbivore–parasitoid interactions are examined in detail.

Acknowledgments We express sincere thanks to Dr. M.D. Rausher for his critical reading of the manuscript. Thanks are also offered to Drs. M. Shimada, C. Godfray, K. Iwao, E. Kuno, and two anonymous reviewers for their valuable advice.

References

Bernays E, Graham M (1988) On the evolution of host specificity in phytophagous arthropods. Ecology 69:886–892

Chew FS (1975) Coevolution of pierid butterflies and their cruciferous food plants. I. The relative quality of available resources. Oecologia 20:117–127

Clausen CP (1940) Entomophagous insects. MacGraw-Hill, New York

Courtney SP (1981) Coevolution of pierid butterflies and their cruciferous food plants. I. The relative quality of available resources. Oecologia 51:91–96

Feltwell J (1981) Large white butterfly: the biochemistry and physiology of *Pieris brassicae* (Linnaeus). Series Entomologia, vol 18. W Junk, The Hague

Iwao K, Ohsaki N (1996) Inter- and intraspecific interactions among larvae of specialist and generalist parasitoids. Res Popul Ecol 38:265–273

Iwao K, Sato Y, Ohsaki N (1989) Spatio-temporal distribution patterns of two tachinid flies, *Epicampocera succincta* and *Compsilura concinnata*, parasitizing *Pieris*. Res Popul Ecol 31:105–112

Jermy T, Szentesi A (1983) The role of inhibitory stimuli in the choice of oviposition site by phytophagous insects. Entomol Exp Appl 24:458–471

Kearney JN (1983) Selection and utilization of natural substance as breeding sites by woodland *Drosophila* spp. Entomol Exp Appl 33:63–70

Nagasaka K (1992) Why does only *Athalia japonica* enter summer diapause among three sympatric *Athalia* sawflies feeding on crucifers? Res Popul Ecol 34:383–395

Nagashima M (1933) Ecology of brachonid wasp, *Apanteles glomeratus* (in Japanese). J Jpn Appl Anim 5:17–25

Ohsaki N (1979) Comparative population studies of three *Pieris* butterflies, *P. rapae*, *P. melete* and *P. napi*, living in the same area. I. Ecological requirements for habitat resources in the adults. Res Popul Ecol 20:278–296

Ohsaki N (1980) Comparative population studies of three *Pieris* butterflies, *P. rapae*, *P. melete* and *P. napi*, living in the same area. II. Utilization of patchy habitats by adults through migratory and non-migratory movements. Res Popul Ecol 22:163–183

Ohsaki N (1982) Comparative population studies of three *Pieris* butterflies, *P. rapae*, *P. melete* and *P. napi*, living in the same area. III. Difference in the annual generation numbers in relation to habitat selection by adults. Res Popul Ecol 24:193–210

Ohsaki N (1986) Body temperatures and behavioral thermoregulation strategies of three *Pieris* butterflies in relation to solar radiation. J Ethol 4:1–9

Ohsaki N, Sato Y (1990) Avoidance mechanisms of three *Pieris* butterfly species against the parasitoid wasp *Apanteles glomeratus*. Ecol Entomol 15:169–176

Ohsaki N, Sato Y (1994) Food plant choice of *Pieris* butterflies as a trade-off between parasitoid avoidance and quality of plants. Ecology 75:59–68

Price PW, Bouton CE, Gross P, McPherson BA, Thompson JN, Weis AE (1980) Interactions among three trophic levels: influence on plants on interactions between insect herbivores and natural enemies. Annu Rev Ecol Syst 11:41–65

Rausher MD (1980) Host abundance, juvenile survival, and oviposition preference in *Battus philenor*. Evolution 34:342–355

Rausher MD (1983) Alteration of oviposition behavior by *Battus philenor* butterflies in response to variation in host-plant density. Ecology 64:1028–1034

Richards OW (1940) The biology of the small white butterfly (*Pieris rapae*), with special reference to the factors controlling its abundance. J Anim Ecol 9:243–288

Roininen H, Tahvanainen J (1989) Host selection and larval performance of two willow feeding sawflies. Ecology 70:129–136

Rowell CHF (1985) The feeding biology of a species-rich genus of rainforest grasshoppers (*Rhachicreaga*: Orthoptera, Acrididae). II. Foodplant preference and its relation to speciation. Oecologia 68:99–104

Sato Y (1976) Experimental studies on parasitization by *Apanteles glomeratus* L. (Hymenoptera: Braconidae). I. Parasitization to different species of genus *Pieris*. Appl Entomol Zool 11:165–175

Sato Y, Ohsaki N (1987) Host-habitat location by *Apanteles glomeratus* and effect of food-plant exposure on host parasitism. Ecol Entomol 12:291–297

Shirozu T, Hara A (1971) Early stages of Japanese butterflies in color. I. Hoikusha, Osaka

Singer MC (1971) Evolution of food-plant preference in the butterfly, *Euphydryas editha*. Evolution 25:383–389

Smiley JT (1978) Plant chemistry and the evolution of host specificity: new evidence from *Heliconius* and *Passiflora*. Science 201:745–747

Strong DR Jr, Lawton JH, Southwood TRE (1984) Insects on plants: community patterns and mechanisms. Blackwell, Oxford

Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol Exp Appl 47:3–14

Wiklund C (1975) The evolutionary relationship between adult oviposition preference and larval host plant range in *Papilio machaon* L. Oecologia 18:185–197

Yano S, Ohsaki N (1993) Phenology and intrinsic quality of wild crucifers that determine the community structure of their herbivorous insects. Res Popul Ecol 35:151–170

Yasumatsu K, Watanabe C (1964) A tentative catalogue of insect natural enemies of injurious insects in Japan. Part 1. Parasitic-predator host catalogue (in Japanese). Entomol Lab Fac Agric Kyushu Univ Fukuoka