Conspecific thistle plant selection by a herbivorous ladybird beetle, *Epilachna pustulosa*

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Abstract

Host selection by *Epilachna pustulosa* Kôno (Coleoptera: Coccinellidae) was surveyed in an area of about 130 ares, focusing on the role of the spatial distribution pattern of the host plant, thistle *Cirsium kamtschaticum* Ledeb. (Asteraceae) and the environmental conditions of habitats where thistle plants were growing. A total of 198 thistle clones were found in the area studied, and approximately 40% showed some degree of *E. pustulosa* infestation by July. Eggs were only oviposited on thistle clones that were fed on by adults. Adult beetles and egg masses of *E. pustulosa* showed an aggregated distribution in the earlier season (June) among thistle clones. The distribution of adults became more random (but still aggregated) by the later season (July), along with an increase in the number of infested clones. Multiple logistic regression analyses revealed that clone size and soil moisture were consistently important for the beetle's choice of clones to feed on. The other logistic regression analyses indicated that thistle-clone size and sunlight condition influenced egg distribution. Thistle clone selection by *E. pustulosa* changed with season from a rather strict selection in June to a more obscure one in July, expanding the range of thistle clones used as feeding and oviposition substrate.

Introduction

Over the past two decades, it has become evident that plant resources for phytophagous insects are much more heterogeneous than was initially recognized (e.g., Crawley, 1983; Denno & McClure, 1983), even among conspecific plants. Hence, phytophagous insects face the necessity of discriminating within and among plant species (Mackay, 1985). The selection of appropriate conspecific plants can be affected by variations in leaf quality (e.g., Whitham, 1983; Karban, 1989; Suomera & Nilson, 1994; Craig et al., 1999, 2000) and other ecological factors, such as the abundance or spatio-temporal distribution of plants (e.g., Rausher & Papaj, 1983; Forsberg, 1987; Brommer & Fred, 1999), the structure of the plants' habitat (e.g., Courtney, 1984; Karban, 1997; Ballabeni et al., 2001), and the composition of competitors and predators on the plants or in the habitat (Price et al., 1980).

Phytophagous insects often have a low mobility in their immature stages, and thus have little opportunity to change the location of their development. For this reason, host plant selection by ovipositing females has the greatest impact on the fate of the offspring (Renwick, 1989; Mayhew, 1997). Therefore, in order to understand the ecological and evolutionary aspects of insect-plant relationships, it is imperative that we investigate host selection by females under natural conditions. One would expect that females will select host plants on which their offspring perform best, even though such a selection process has not always been supported by empirical studies (reviewed in Thompson, 1988; Courtney & Kibota, 1990; Jaenike, 1990; Thompson & Pellmyr, 1991; Leather, 1994; Mayhew, 1997). Females may not always select the optimal host for their offspring, depending on their own physiological state, the abundance of optimal and suboptimal hosts, and a density-dependent response (reviewed in Jaenike, 1990; Mayhew, 1997). For example, females could begin to oviposit on suboptimal hosts with an increase of age or egg-load. Therefore, the manner of host selection may change over time, and the consideration of seasonal changes in host selection patterns could be of

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particular importance in understanding insect-plant relationships.

Previously, we reported that considerable individual variation in leaf quality exists in the thistle *Cirsium kamtschaticum*, the host plant of the herbivorous ladybird beetle *Epilachna pustulosa* (Fujiyama & Katakura, 1997). When leaves sampled from the field were examined in the laboratory, the thistle showed qualitative intraspecific variation which affected both adult female feeding preference and larval performance of *E. pustulosa*. A series of subsequent experiments that dealt with the transplantation of several thistle plants into a common garden and the manipulation of sunlight intensity to thistle leaves revealed that the intraspecific variation of thistle is caused by both genetic and environmental factors (Fujiyama & Katakura, 2001). Basically, adult females appear to prefer plants on which larval performance is good (Fujiyama & Katakura, 1997), but in some cases they may choose plants less adequate for larval growth, particularly in habitats with unstable environmental conditions (Fujiyama & Katakura, 2001).

These laboratory results do not always explain host selection of *E. pustulosa* under natural conditions (N. Fujiyama, unpubl.). In our preliminary study, *E. pustulosa* in a natural environment sometimes differentially utilized thistle plants that were found to have a similar leaf quality in laboratory experiments. Hence, other unidentified ecological factors, as well as the variation in leaf quality among thistle plants, must play an important role in host selection by *E. pustulosa* under natural conditions.

In the present study we surveyed host selection by *E. pustulosa* under natural conditions, focusing in particular on the role of the spatial distribution pattern of thistle plants and the environmental conditions of habitats where thistle plants were growing. Particular questions examined were as follows: (i) When the spatial and the environmental characteristics are regarded as attributes of each thistle individual, which plant is more likely to be infested by *E. pustulosa*? (ii) Does thistle plant selection by *E. pustulosa* change seasonally, and if so, how?

Materials and methods

Insects and plants

Epilachna pustulosa is a univoltine coccinellid species endemic to Hokkaido, northern Japan (Katakura, 1981). In the Sapporo area, post-hibernating adults appear in early May and begin oviposition in late May Oviposition continues until July. Females lay eggs in masses, usually on the underside of the leaves of host plants. Emergence of larvae starts in early June. Adults and larvae feed on the same plant species. New adults emerge from early August and enter hibernation by late September.

Epilachna pustulosa occurs mainly on the thistle, *Cirsium kamtschaticum*. In and near the Sapporo area, *E. pustulosa* also occurs on blue cohosh, *Caulophyllum robustum* Maxim. (Berberidaceae), when this plant grows together with thistles (Kurosawa, 1953; Katakura, 1974, 1981).

Cirsium kamtschaticum is a perennial wild herb and is the most abundant and prevailing species of thistle in Hokkaido (Kadota, 1995). This thistle species grows nearly everywhere in forest margins, along streams, and in grassy fields, as an assemblage of a few to more than 15 clonal shoots. These assemblages can be considered as individual units that can be easily distinguished with the naked eye. Hereafter, we call each unit a 'clone' (cf. Fujiyama & Katakura, 1997), although different units may not necessarily possess different genotypes. The general phenology of *C. kamtschaticum* in the Sapporo area is as follows: most clones sprout in late April, soon after the disappearance of the snow cover. All shoots within a clone emerge and grow almost simultaneously. Shoots grow rapidly to about 2 m in height by June, and bloom from June to July. Shoots wither by mid-August, while rosettes grow from July and remain until early October

Because the larvae of *E. pustulosa* have a low mobility, their development is usually restricted to the plant or a cluster of plants on which they were laid as eggs. When laid on *C. kamtschaticum*, *E. pustulosa* larvae basically cannot move from one clone to another. On the other hand, the larvae can move between shoots within a single clone, because the leaves of different shoots of the same *C. kamtschaticum* clone often touch.

Area studied

We selected an area of about 130 ares at Hoshioki, a suburb west of Sapporo. The area was composed of various environment types, including damp areas, grassland, and groves, and consisted mainly of the following three habitats (Figure 1).

1 A narrow damp grove belonging to a nature reserve of the skunk cabbage, *Lysichiton camtschatcense* (L.) Schott. This habitat had low light intensity and was very damp.

2 A windbreak artificial grove along a railway line. This habitat also had low light intensity, but its soil was relatively dry.

3 Sunny open grassland stretching along a railway line, adjacent to a natural grove that had developed on a steep slope facing north. The soil of this habitat was relatively dry.

Many clones of the thistle *C. kamtschaticum* were growing throughout the area, and *E. pustulosa* occurred on those plants. Prior to conducting the censuses (see below), we identified all the *C. kamtschaticum* clones growing in the area, marked them with plastic tags, and mapped them.

A few clones of another recently naturalized thistle species, *Cirsium vulgare* (Savi) Tenore, and two large patches

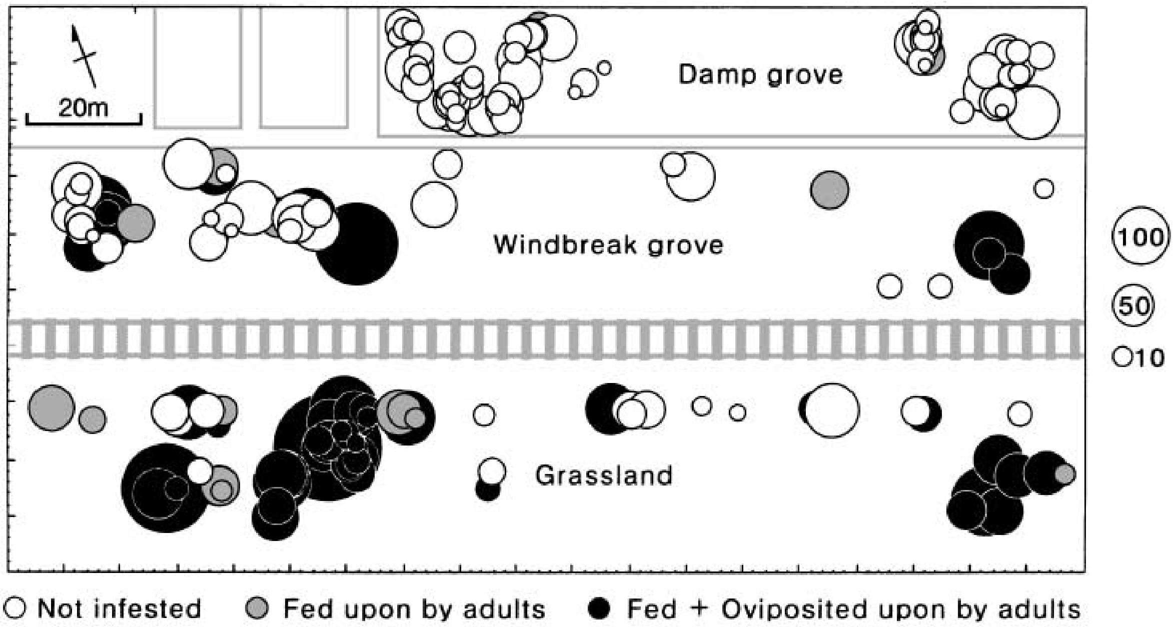


Figure 1 Spatial distribution of thistle *Cirsium kamtschaticum* clones in the area surveyed. Each circle represents a thistle clone. Differences in the clone size (calculated as leaf dry weight, g) and the categories of infestation by *Epilachna pustulosa* [for the second (July) census] are indicated as differences in the sizes and colors of circles, respectively.

of blue cohosh were growing in the study area and adjacent to the southern boundary of the study area, respectively. Although these plants were also often fed upon by *E. pustulosa* (Yamaga & Ohgushi, 1999; Fujiyama & Katakura, 2002), the possible influence of these plants on the utilization of *C. kamtschaticum* by *E. pustulosa* was not taken into account in the present study.

Censuses

The main census was carried out twice in 1997, once in early June when oviposition by *E. pustulosa* started, and again in late June to early July when oviposition was most frequent. Hereafter, these two censuses are referred to as the June and July censuses, respectively. Environmental factors, which could be considered to be rather stable, irrespective of seasons and from year to year, were measured in August 1998.

Infestation by *Epilachna pustulosa* on thistle clones

In the June and July censuses, we checked the number of *E. pustulosa* adults on each thistle clone for each sex. The presence/absence of egg masses and larvae, and the feeding traces of adult beetles were also checked. Because the mobility of *E. pustulosa* larvae is very low (see above), the presence of larvae on a particular clone was treated as evidence of oviposition on the clone, even if eggs or eggshells were not found. The number of egg masses on each thistle clone was further counted only in the June census, as an accurate count of the cumulative number of egg masses later in the season was impossible due to hatching and the subsequent falling off of the eggshells.

Characteristics of thistle clones and habitats

We estimated the following characteristics of thistle clones and environmental conditions of the three habitats.

Size of thistle clones. In July 1997, we measured the volume (V cm^3: height × the broadest breadth × the narrowest breadth) of all shoots composing each thistle clone. The measurements took about 1 week; the disparity of clone size caused by this time lag was adjusted using the value of a control clone that was measured at the beginning and end of the measurements, assuming that the thistle clones grew linearly during the census period. The size of each clone was expressed as the dry weight of total leaves, which could be estimated from the volume of the shoot (Hinomizu et al., 1981) using the following equations obtained through our preliminary study in the area. In these equations, W_L and W are the dry weight of total leaves of the shoot (g) and the total dry weight of the shoot (g), respectively.

$$W_L = 0.369\,W\ (r = 0.98, P < 0.001)$$

$\log W = 1.133 \log V - 4.860$ ($r = 0.90$, $P < 0.001$)

Conspecific abundance of thistle clones. The distance of dispersal of *E. pustulosa* adults has been reported to be about 1 m per day (Kimura & Katakura, 1986). Therefore, to evaluate the conspecific abundance of each thistle clone, we used the field map and defined a 'patch' as an assemblage of thistle clones growing less than 1 m from each other. In the present study, the conspecific abundance was expressed as the dry weight of total leaves of all clones (see above) other than the clone being focussed upon within a given patch. For example, the conspecific abundance of a thistle clone without any other clones within a radius of 1 m would be calculated as zero by this method. This parameter thus reflects the spatial distribution pattern or patchiness of thistle clones, but is independent of the size of the clone being studied.

Relative illumination of habitat. To estimate sunlight conditions in the area, we divided the area into 128 quadrates of about 100 m^2 each. The relative illumination (%) of each quadrate was calculated as the illumination of each quadrate relative to that of the nearest open site. Illumination was measured with a portable illuminometer (IM-3; Topcon, Tokyo, Japan) in 1998. The relative illumination of each quadrate was applied to all the clones growing in the quadrate as the value for each clone.

Ground temperature and soil moisture of the habitat. Another set of 14 quadrates (about 930 m^2 each) was established to measure ground temperatures and soil moisture. Ground temperature at a depth of 10 cm below the soil surface was measured at each quadrate. The disparity of temperature caused by the time lag of a series of measurements was adjusted using the value of a control quadrate that was measured at the beginning and end of the measurements, assuming that the temperature changed linearly during the census period. At each quadrate, about 30 ml of topsoil was sampled and was brought back to the laboratory. To calculate soil moisture, soil samples were weighed after sifting through a sieve (1 mm cross), and weighed again after drying in an oven at 80 °C for more than 48 h. As with sunlight conditions, the ground temperature and soil moisture of each quadrate were applied to all clones growing in the quadrate. Soil sampling was carried out simultaneously with the measurement of temperatures in 1998, on a clear day after at least three continuous days without rain.

Analyses of ladybird distribution

To describe the distribution pattern of *E. pustulosa* adults and egg masses on thistle clones, we used the concept of 'mean crowding' (Lloyd, 1967; Iwao, 1977; Southwood & Henderson, 2000). All the parameter expressions followed those of Southwood & Henderson (2000).

First, mean crowding ($\dot{x}$), the mean number of other conspecific individuals per individual per unit, was calculated by the following formula with mean density per unit ($\bar{x}$) and variation among units (s^2).

$$\dot{x} \approx \bar{x} + \left(\frac{s^2}{\bar{x}} - 1\right)$$

The ratio of mean crowding to mean density ($\dot{x}/\bar{x}$) provides a relative measure of aggregation, i.e., a degree of departure from randomness of the observed pattern (Iwao, 1977). It equals unity in the random (Poisson) distribution, and is greater and smaller than unity in the contagious (aggregated) and regular (uniform) distributions, respectively. The significance of departure from random distribution was examined by two-tailed f-test using $s^2/\bar{x}$ as the subjective F-value, $n - 1$ as the degree of freedom (n is the total number of units) for the numerator, and infinity as the degree of freedom for the denominator (Ito et al., 1992). In the present study we treated each thistle clone as a unit.

Examination of responsible factors for occurrence of infestation

To examine the putative effect of thistle clone size, conspecific thistle abundance, relative illumination, soil moisture, and ground temperature on the occurrence of feeding/oviposition by *E. pustulosa* on thistle clones, we conducted a maximum likelihood estimation in multiple logistic regression using statistical analysis software (JMP ver. 3.2.2; SAS Institute Inc., Cary, NC). We employed this procedure because the distribution of the presence or absence of infestation by *E. pustulosa* was binomial rather than normal. The importance of each factor was evaluated by comparing the value of the loss of function between the complete model and the model with that factor removed.

Results

In the area studied, we found 198 thistle clones composed of 318 shoots. Of these, 41.9% (83 clones) were infested by *E. pustulosa* to some extent by July. Fifteen clones could not be found in the second census or had unfortunately been cut down between the two censuses, and nine clones were newly found in the second census. The occurrence of ladybird infestation was most frequent in the sunny open grassland located in the southern part of the area examined (at least 78.4% of 74 clones were infested to some extent by July), followed by the windbreak grove located in the middle of the area examined (at least 38.6% of 57 clones). In the damp grove located in the northern part, the

Table 1 Calculated mean crowding ($\dot{x}$) and $\dot{x}/\bar{x}$ value for distribution of *Epilachna pustulosa* beetles among thistle *Cirsium kamtschaticum* clones. The other statistics (d.f. and F) for testing departure from random distribution (Ito et al., 1992) are also given

	June				July			
	$\dot{x}$	$\dot{x}/\bar{x}$	d.f.	F ($s^2/\bar{x}$)	$\dot{x}$	$\dot{x}/\bar{x}$	d.f.	F ($s^2/\bar{x}$)
Female	0.9	3.51	188,∞	1.655***	0.3	2.38	181,∞	1.183
Male	2.0	9.07	188,∞	2.778***	0.8	4.32	181,∞	1.574***
Both sexes pooled	2.7	5.53	188,∞	1.854***	1.2	3.71	181,∞	1.854***

***$P < 0.001$.

frequency of ladybird infestation was very low (4.5% of 67 clones) (Figure 1). Eggs were only found to have been oviposited on thistle clones fed upon by *E. pustulosa* adults.

Distribution pattern of ladybirds

Adult beetles of *E. pustulosa* showed an aggregated distribution among thistle clones in both censuses; the distribution patterns almost always departed significantly from a random distribution, and the $\dot{x}/\bar{x}$ values were larger than 1 in all cases (Table 1). The $\dot{x}/\bar{x}$ values for the July census were consistently smaller than those for the June census (Table 1), indicating that the distribution of adult beetles changed from a aggregated to a random distribution with season.

Egg masses of *E. pustulosa* were also aggregated among thistle clones; the distribution pattern departed significantly from random distribution (∞, F = 6.13, d.f. = 188, $P < 0.0001$), and the $\dot{x}/\bar{x}$ value was 10.46 with a mean crowding ($\dot{x}$) of 5.7.

Contribution of thistle and environmental factors to ladybird infestation

As described above, eggs were only found to have been oviposited on thistle clones fed upon by *E. pustulosa* adults. Hence, in the analyses, we classified thistle clones into the following three categories: NI, not infested by *E. pustulosa* at all; F, fed but not oviposited upon by adult beetles; FO, fed and oviposited upon by adults. The percentages of clones belonging to each category are shown in Table 2. Multiple logistic regression analyses were conducted for (1) clones fed upon and clones not infested [between (F + FO) and NI], and (2) fed clones oviposited and fed clones not oviposited (between FO and F).

Table 2 Percentage (n) of thistle *Cirsium kamtschaticum* clones belonging to each category of infestation by *Epilachna pustulosa*

	Infestation category[a]		
	NI	F	FO
June census	65.7 (113)	16.3 (28)	18.0 (31)
July census	58.6 (106)	9.4 (17)	32.0 (58)

[a] NI, not infested; F, fed but not oviposited upon by adults; FO, fed and oviposited by adults.

The occurrence of feeding by *E. pustulosa* adults was significantly regressed with all parameters for both the June ($\chi^2 = 89.56$, d.f. = 5, $P < 0.0001$ with 172 clones) and July ($\chi^2 = 100.48$, d.f. = 5, $P < 0.0001$ with 181 clones) censuses. In the June census, the effects of clone size, conspecific thistle abundance, soil moisture, and ground temperature were significant (Table 3a): larger thistle clones having greater conspecific abundance, growing in habitats with relatively dry soil and with lower ground temperature were more likely to be fed upon (Figure 2). In the July census, the effects of clone size and soil moisture were significant (Table 3a): larger thistle clones and those growing in habitats with relatively dry soil were more likely to be fed upon (Figure 2).

The occurrence of oviposition by *E. pustulosa* was also significantly regressed with all parameters for both the June ($\chi^2 = 23.09$, d.f. = 5, $P = 0.0003$ with 59 clones) and July ($\chi^2 = 23.52$, d.f. = 5, $P = 0.0003$ with 75 clones) censuses. In the June census, the effects of clone size and relative illumination were significant (Table 3b): among clones fed upon by adults, larger clones, and those growing in sunnier habitats were more likely to be oviposited upon (Figure 3). In the July census, only the effect of clone size was significant (Table 3b): larger clones were more likely to be oviposited upon (Figure 3).

Discussion

Feeding pattern of *Epilachna pustulosa* adults

Adult beetles of *E. pustulosa* showed an aggregated distribution among thistle clones (Table 1). This indicates that infestation by *E. pustulosa* tended to occur on particular thistle clones within the area studied. In the June census, feeding by *E. pustulosa* adults was likely to occur on larger thistle clones and those with greater conspecific abundance, growing in habitats with relatively dry soil and lower ground temperature (Table 3a, Figure 2). The effects of conspecific thistle abundance and ground temperature

Table 3 Multiple logistic regression tables for the effects of several factors on the occurrence of adult feeding and oviposition by *Epilachna pustulosa* on thistle *Cirsium kamtschaticum* clones

(a) Occurrence of adult feeding for all clones

		June census (n = 172)			July census (n = 181)		
Factor	d.f.	Coefficient	χ^2	P	Coefficient	χ^2	P
Intercept	1	33.976			19.178		
Clone size	1	0.025	13.360	< 0.001	0.025	12.054	< 0.001
Conspecific thistle abundance	1	0.011	4.069	0.044	0.005	1.310	NS
Relative illumination	1	0.010	3.212	NS	0.009	2.612	NS
Soil moisture	1	−0.052	16.714	< 0.001	−0.070	36.924	< 0.001
Ground temperature	1	−1.603	8.324	0.004	−0.821	2.587	NS

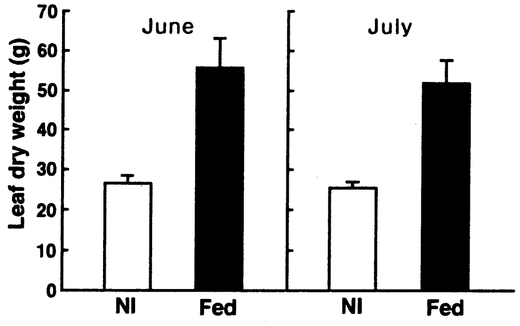
(b) Occurrence of oviposition for clones that were fed upon by adults

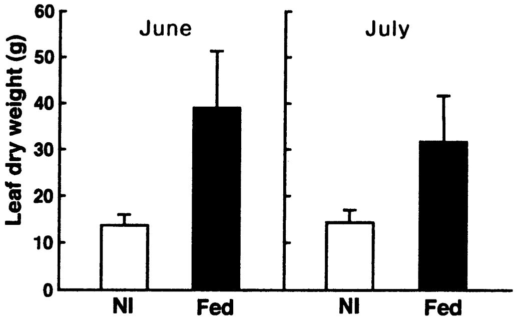
		June census (n = 59)			July census (n = 75)		
Factor	d.f.	Coefficient	χ^2	P	Coefficient	χ^2	P
Intercept	1	−0.103			−12.199		
Clone size	1	0.034	13.957	< 0.001	0.038	8.662	0.003
Conspecific thistle abundance	1	−0.004	1.648	NS	0.045	3.645	NS
Relative illumination	1	0.021	6.764	0.009	0.014	2.824	NS
Soil moisture	1	−0.016	0.132	NS	−0.067	3.622	NS
Ground temperature	1	−0.087	0.006	NS	0.675	0.710	NS

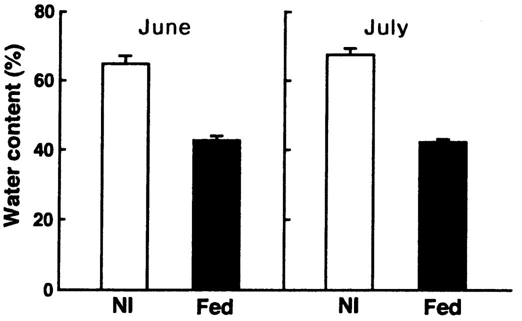
became less important in the July census (Table 3a). With the change in season, the distribution of adult beetles changed from an aggregated to a random distribution (Table 1), and this change was concomitant with an increase in the number of clones fed upon (Table 2). It is assumed that, later in the season, adult beetles began to select thistle clones that were not initially utilised. The increase in the number of clones fed upon made the selection by *E. pustulosa* adults appeared to be less strict than that in the earlier season. The difference between the mean values for clone size, conspecific thistle abundance, and the ground temperature of clones not infested and clones fed upon was somewhat reduced over time (Figure 2).

The observed pattern of ladybird beetle infestation may include two different components of host selection, i.e., selection of a particular habitat type, and selection of a particular thistle clone (cf. Courtney, 1984). In the present study, *E. pustulosa* consistently selected larger thistle clones (clones with greater conspecific abundance were not always selected) and consistently did not select clones growing in the very damp habitat (Table 3a, Figure 2). These results appear to reflect the rarity of *E. pustulosa* infestation in the northern damp grove (Figure 1). In our previous laboratory experiments, adult females of *E. pustulosa* preferred leaves of clones growing in the southern open grassland to leaves of clones growing in the northern damp grove (cf. Fujiyama & Katakura, 1997). This may, at least in part, explain the rarity of infestation in the damp grove. However, in the present study, it is not likely that the paucity of infestation in the grove was the result of the direct rejection of thistle clones by adults, because neither adult beetles nor feeding traces of *E. pustulosa* were found in substantial numbers on the thistle clones growing there. It is probable that *E. pustulosa* avoided a proportion of thistle clones through the selection of a particular habitat type. It is noteworthy that, when a further regression analysis with only the thistle clones growing in the southern grassland (the habitat in which the ladybird infestation was most frequent; Figure 1) was conducted for the June census ($\chi^2 = 20.90$, d.f. = 5, P = 0.0008 with 63 clones), the effect of soil moisture was not significant (Table 4).

On the other hand, the importance of clone size and conspecific thistle abundance was still obvious when analysis was restricted to the clones growing in the southern grassland (Table 4). Our previous experiments suggested that adult females of *E. pustulosa* did not show a clear feeding preference among thistle clones growing in this habitat (cf. Fujiyama & Katakura, 1997). Therefore, it can be concluded that ecological factors such as clone size or conspecific abundance of thistle, as well as the variation in leaf quality among thistle clones (Fujiyama & Katakura, 1997, 2001), plays an important role in thistle clone selection by *E. pustulosa* under natural conditions.


a) Clone size


b) Conspecific thistle abundance


c) Soil moisture

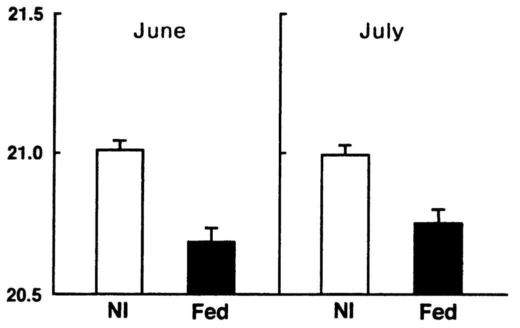
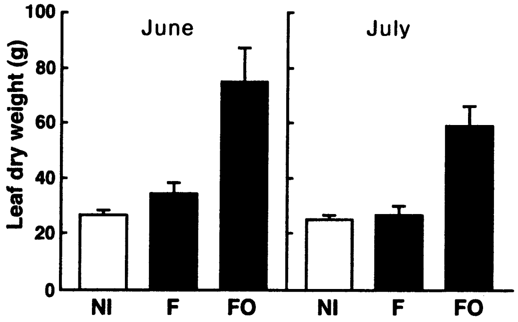

d) Ground temperature (°C)

Figure 2 Mean + SE for the factors related to the occurrence of feeding by *Epilachna pustulosa* adults on thistle *Cirsium kamtschaticum* clones. NI, not infested at all; Fed, fed upon by adults. Sample sizes (n) for the two censuses are: June, NI, 113; June, Fed, 59; July, NI, 106; July, Fed, 75.


a) Clone size

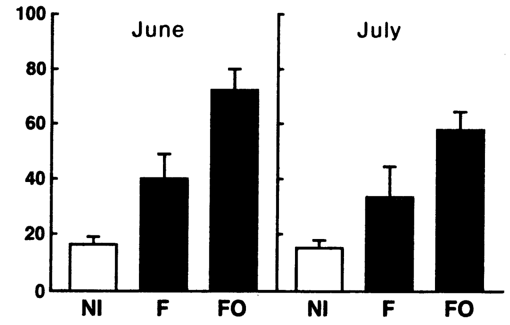

b) Relative illumination (%)

Figure 3 Mean + SE for the factors related to the occurrence of oviposition by *Epilachna pustulosa* on thistle *Cirsium kamtschaticum* clones. NI, not infested at all; F, fed but not oviposited upon by adults; FO, fed and oviposited upon by adults. Sample sizes (n) for the two censuses are: June, NI, 113; June, F, 28; June, FO, 31; July, NI, 106; July, F, 17; July, FO, 58.

One possible ecological determinant of the distribution of *E. pustulosa* when post-hibernating adults appear, is the hibernation site of the previous year. Just before entering hibernation in autumn, newly emerged adults usually feed on rosettes of a few available thistle plants (Ehara, 1952; Hinomizu, 1976; Katakura, 1976, 1981; Hinomizu et al., 1981; Kimura & Katakura, 1986). It is likely that these adults enter hibernation in the vicinity of the thistle clones they feed upon. Therefore, adequacy as hibernation site would have a strong influence in the initial phase of both habitat and thistle clone selection by *E. pustulosa*. Further studies will be needed to elucidate the effect of hibernation site and the ability and pattern of individual movement of beetles, and thereby to clarify thistle plant selection by *E. pustulosa* under natural conditions.

Selection of larval food by *Epilachna pustulosa*

Egg masses of *E. pustulosa* also showed an aggregated distribution among thistle clones, indicating that oviposition by *E. pustulosa* tended to occur on particular

Table 4 Multiple logistic regression table for the effects of several factors on the occurrence of adult feeding by *Epilachna pustulosa* on thistle *Cirsium kamtschaticum* clones growing in the southern grassland. Results for the June census are shown (n = 63)

Factor	d.f.	Coefficient	χ^2	P
Intercept	1	3.196		
Clone size	1	0.035	7.139	0.008
Conspecific thistle abundance	1	0.450	6.089	0.014
Relative illumination	1	0.011	2.321	NS
Soil moisture	1	–0.013	0.011	NS
Ground temperature	1	–0.199	0.012	NS

thistle clones. In the June census, of the clones fed upon by adults, larger thistle clones and those growing in sunny habitats were most likely to be oviposited upon (Table 3b, Figure 3). Then, in the July census, the effect of relative illumination became obscure (Table 3b), with an increase in both the number and rates of clones oviposited upon (Table 2). The pattern of seasonal change in oviposition was similar to the feeding pattern; the increase in the number of clones oviposited upon made the selection by females appear to be less strict. Both for clone size and relative illumination, the difference between the mean values of clones not oviposited upon and those oviposited upon decreased in time (Figure 3).

As with the feeding patterns mentioned above, there is a possibility that the observed pattern of oviposition may include both habitat selection and thistle clone selection. However, because the regression analyses for oviposition pattern (Table 3b) were conducted among clones fed upon, the possible effects of habitat or hibernation site selection were considered to play no more than a negligible role in the analysis of oviposition selection (Table 3b, Figure 3).

The decision by *E. pustulosa* females to select thistle clones growing in sunny habitats as their oviposition sites appears to be adaptive, as the leaves of thistle clones in such habitats are generally more suitable as larval food; in previous laboratory experiments, the larval eclosion rates of *E. pustulosa* increased significantly when the larvae were reared on thistle leaves exposed to higher sunlight intensities (Fujiyama & Katakura, 2001). On the other hand, oviposition on larger thistle clones could occur somewhat accidentally, due to the manifestation of these clones with respect to visual or olfactory cues. Nonetheless, the selection of larger clones may be advantageous, as it ensures an abundance of larval food. To investigate the adaptive significance of the oviposition pattern of *E. pustulosa*, more data on larval performance under various natural conditions should be gathered in future studies.

The present study focused particularly on the factors correlated with the 'occurrence' of oviposition and feeding, and there must be other factors that act as determinants of egg densities on thistle plants. For example, Kimura & Katakura (1986) suggested that the number of eggs laid on thistle plants depends not on the size of individual clones but on the size of thistle patches (referred to as subpatches).

Seasonal changes in thistle clone selection by *Epilachna pustulosa*

In the present study, thistle clone selection by *E. pustulosa* adults for both feeding and oviposition changed from highly selective to less selective over time (Tables 2 and 3, Figures 2 and 3). The seasonal change may be a consequence of a density-dependent process (cf. Mayhew, 1997). As was strongly suggested in a study of the closely related congeneric species *E. niponica* Lewis (Ohgushi, 1999), a density-dependent dispersal process, such as increased female movement in parallel with increased egg densities, can be expected in *E. pustulosa* (Y. Yamaga, pers. comm.). It is probable that the preceding feeding and/or oviposition by conspecifics reduced the relative suitability of thistle clones, and then *E. pustulosa* began to select clones that were initially regarded as less suitable based on the criteria examined in the present study. Under natural conditions, *E. pustulosa* may select the most appropriate thistle clones in the respective seasons and environments.

There have been several studies on the host selection of *E. pustulosa* under natural conditions (Hinomizu, 1976; Katakura, 1976; Kimura & Katakura, 1986; Yamaga & Ohgushi, 1999). Even though some authors have noted the importance of plant size or hibernation site, heterogeneity within the host plant species has seldom been taken into account. We emphasize that both the variable properties among conspecific plants and the differences between plant species play an important role in shaping insect-plant relationships.

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