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Distribution and host specificity of the thistle-feeding tortoise beetle *Cassida vibex* (Coleoptera: Chrysomelidae) in southwestern Hokkaido, northern Japan

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*Running head: Cassida vibex* distribution in Hokkaido

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## Abstract

The thistle-feeding tortoise beetle *Cassida vibex* (Coleoptera: Chrysomelidae) is widespread in the Palearctic region. In Japan, this species has been recorded only from the largest island, Honshu. Here we report the occurrence of this species on Hokkaido, the most northern main island of Japan, along with detailed distributional records from the southwestern part of the island (southern Oshima Peninsula) and information on host plants. We also present the results of laboratory experiments on adult feeding preference and larval developmental performance to determine the specificity of *C. vibex* for four thistle species (*Cirsium grayanum*, *Cir. alpicola*, *Cir. yezoense*, and *Cir. aomorense*; Asteraceae) common in southwestern Hokkaido. *Cassida vibex* was detected at only four sites among 97 sites investigated on the Oshima Peninsula, in strong contrast to the common occurrence of the congeneric thistle feeder *C. rubiginosa* (74 of 97 sites). *Cassida vibex* was found mainly on *Cir. alpicola* in the wild, but adult beetles showed no feeding preference for this or three other thistle species when given a choice in the laboratory. Larval performance of *C. vibex* was similar on *Cir. alpicola*, *Cir. yezoense*, and *Cir. aomorense* but significantly lower on *Cir. grayanum*. Since *Cir. alpicola* often forms large clumps of individual plants, whereas *Cir. yezoense* and *Cir. aomorense* are scattered in their habitats, and *C. vibex* was collected only at sites where there were dense patches of *Cir. alpicola*, we postulate that the persistence of *C. vibex* is possible only where a substantial amount of suitable host plants is available.

**Key words:** adult feeding preference, *Cassida rubiginosa*, *Cirsium* spp., insect-plant relationships, larval developmental performance.

## INTRODUCTION

The geographic distribution of phytophagous insect species is determined by various abiotic and biotic factors. Among these factors, the occurrence of potential host plants in a given habitat or region may be the most crucial proximate factor (Quinn *et al.* 1997, 1998; Koizumi *et al.* 1999; reviewed in Janz & Nylin 2008), because species of phytophagous insect are more or less host specific (Strong *et al.* 1984; Bernays & Chapman 1994; Schoonhoven *et al.* 2005). The distribution of phytophagous specialists is expected to be more strongly restricted by the availability and abundance of suitable host plants than that of phytophagous generalists (Root 1973; Quinn *et al.* 1997, 1998; see also Päävinen *et al.* 2003).

The thistle-feeding tortoise beetle *Cassida vibex* L. (Coleoptera: Chrysomelidae) is distributed in the Palearctic region, including Mongolia, northern China, Siberia, Europe, and Japan (Chûjô & Kimoto 1961; Hayashi *et al.* 1984; Chen *et al.* 1986; Hirashima 1989; Kimoto & Takizawa 1994; Morimoto 2007). Reports in the literature indicate that the distributional range of this beetle in the Japanese Archipelago is restricted to the largest island, Honshu (Chûjô & Kimoto 1961; Hayashi *et al.* 1984; Hirashima 1989; Kimoto & Takizawa 1994; Morimoto 2007). However, several researchers (N. Fujiyama, unpubl. data, 1993; Y. Tomioka, pers. comm., 2003) have found *C. vibex* in southwestern Hokkaido, the most northern of the main islands of Japan. Here we report in detail the distribution of *C. vibex* in the southern part of the Oshima Peninsula, southwestern Hokkaido, as the first record of this beetle from Hokkaido. Concerning the host specificity of European populations of this beetle species, there have been detailed host records in the wild and information on adult feeding preference under laboratory conditions at plant species level (Zwölfer & Eichhorn 1966). For Japanese populations, however, host plant information of *C. vibex* in the wild has been usually roughly described with a common name of a plant group, i.e. 'thistles', which mainly refers to plants of the genus *Cirsium* (Asteraceae) (Chûjô & Kimoto 1961; Hayashi *et al.* 1984; Kimoto & Takizawa 1994; Morimoto 2007), except

for a few fragmentary records during surveys on local fauna (e.g. Tomioka 2000; records on *Cir. nipponicum* (Maxim.) Makino at Aomori Prefecture). In addition, no attempts have been made so far to elucidate the host specificity of Japanese populations of *C. vibex* under laboratory conditions. Hence, in this study, we also examined through field surveys and laboratory tests the ability of *C. vibex* to utilize potential host plants, thistles in the genus *Cirsium*. We discuss factors that may determine the beetle's distribution on the Oshima Peninsula, with special attention to the quality and quantity of host plants available to the herbivorous specialist.

## **MATERIALS AND METHODS**

### **Field survey**

*Cassida vibex* is univoltine and overwinters as adults. Females lay eggs in masses enclosed in oothecae on their food plants, with each ootheca containing a few to several eggs. Both adults and larvae feed on the leaves of thistles (Kimoto & Takizawa 1994). Field surveys were carried out intermittently from spring to autumn (May-September), 2003 through 2006, at 97 sites across the southern part of the Oshima Peninsula and on Okushiri Island (Fig. 1). At each site and on each occasion of field observation, we recorded the species of thistles present and the incidence of *C. vibex* oothecae, larvae, pupae, adults, and feeding scars. Basically, all leaves of all thistle individuals at each site were investigated. At sites with more than 20 thistle individuals, we selected and carefully inspected at most 20 thistle individuals, including all thistle species occurring at the sites. Thistles were identified according to Kadota (1995) and Takita (2001). We also investigated with the same method the incidence of the common, congeneric thistle feeder *C. rubiginosa* Müller. *Cassida vibex* and *C. rubiginosa* are clearly distinguishable by differences in the color of oothecae, old larvae, pupae, and adults, though young larvae and feeding scars look very similar between them. In *C. rubiginosa*, oothecae are deep brown and old larvae and pupae are dark brown, whereas in *C. vibex*,

oothecae are pale brown and old larvae and pupae are light green. The oothecae of *C. vibex* are smaller than those of *C. rubiginosa*.

## **Experiments**

We conducted all experiments under the controlled regime of a 16L:8D cycle at 20°C, using posthibernating adults of *C. vibex* collected on *Cir. alpicola* Nakai at a site at Shiriuchi, Kamiiso (41°37'N, 140°21'E) where the density of the beetle was highest. As plant material, we used the four thistle species common in the southern part of the Oshima Peninsula, namely, *Cir. grayanum* (Maxim.) Nakai, *Cir. alpicola*, *Cir. yezoense* (Maxim.) Makino, and *Cir. aomorense* Nakai (Koizumi *et al.* 1997; also see RESULTS).

### *Adult feeding preference*

We carried out food choice tests in 2005 to assess the adult feeding preference of *C. vibex*. Posthibernating adult beetles used were collected on 8 June 2005. All plant material used in the experiment came from plants transplanted in 2003 and 2004 from a site at Kikonai, Kamiiso (41°44'N, 140°29'E) to an experimental garden on the Hakodate Campus, Hokkaido University of Education (41°47'N, 140°44'E).

Because *C. vibex* occurred mainly on *Cir. alpicola* in the wild (see RESULTS; Table 1), the preference for the other three thistle species relative to *Cir. alpicola* was investigated. Two leaf discs ( $\varnothing$  4 cm, each) from *Cir. alpicola* and one of the other three thistle species were placed in a transparent polystyrene container (8.0 × 15.5 × 3.0 cm), the bottom of which was lined with moistened filter paper. A beetle was released into the container and was allowed to feed on the discs for 24 h. Each beetle was tested twice for each combination of thistle species, on two successive days. Prior to each two-day test, the beetle was fed on *Cir. alpicola* leaves for more than 24 h, in order to standardize the possible influence of preceding feeding experience. In preliminary experiments conducted in 2003, we observed a conspicuous decrease with time in the amount eaten by beetles (K. Togashi, unpublished data). Therefore, the order of

experiments with different combinations of thistle species was randomized among the beetles examined, and all experiments were finished within the shortest period (nine days). Nineteen to 20 adult beetles of each sex were tested with each combination of thistles.

Leaf areas consumed were measured with image processing software (NIH Image ver. 1.63; National Institutes of Health, Bethesda, MD) after the leaf discs were photocopied and scanned into a computer. Differences in thickness or water content between thistle species can create biases in terms of feeding area. To minimize these biases, areas measured were converted to wet weights (mg) by multiplying by thistle species-specific coefficients obtained in our preliminary study. A preference for either thistle species, as determined by the relative amounts consumed during the two-day test, was analyzed by a Student's *t*-test for paired comparisons.

#### *Larval performance*

The larval developmental performance of *C. vibex* on the four thistle species was examined in a rearing experiment in 2003. Egg masses were gathered daily from 10 posthibernating females collected on 22 May 2003 and kept individually in the laboratory. Newly hatched larvae from one egg mass (range 1-4 larvae) were assigned to different thistle species, and a total of 20 larvae derived from respective females were evenly assigned to the four thistle species. Larvae were reared individually in a transparent polystyrene case (5.5 × 6.0 × 1.5 cm), the bottom of which was lined with moistened filter paper. Throughout the experiment, we provided sufficient amounts of middle-aged leaves of food plants that were picked once a week from the Kikonai site and kept at 5°C in a refrigerator.

Larvae were reared until emergence. Larval survival and the occurrence of pupation and adult emergence were recorded daily. The number of individuals emerged on thistle species and the developmental period (number of days from hatching to adult emergence) were recorded for each larva. The pronotum width of emerged adults was

measured under a binocular microscope to the nearest 0.01 mm as an index of body size. The adults were then dissected to determine their sex.

The rearing experiment was conducted again in 2005 with almost the same design as in 2003, with the offspring of the females used in the feeding tests described above. As larval foods in 2005, we used middle-aged thistle leaves from the transplanted thistle individuals that were also used for feeding tests with adult beetles (see above).

To test the effect of thistle species on larval performance, we conducted a logistic regression analysis for survival to adulthood with factors of food, year, and food  $\times$  year interaction, and mixed-model ANOVAs using log-transformed data on developmental period and pronotum width, with factors of sex, food, year, and food  $\times$  year interaction. In the ANOVAs, we regarded year and the food  $\times$  year interaction as random effects, and sex and food as fixed effects. These analyses were carried out using JMP 8.0.2 statistical software (SAS Institute, Cary, NC).

## RESULTS

### Field survey

We found nine thistle species in the area surveyed (Table 1). As a previous study showed (Koizumi *et al.* 1997), *Cir. grayanum* dominated, but *Cir. alpicola*, *Cir. yezoense*, and *Cir. aomorense* were also common in the area. *Cirsium pendulum* Fisch. ex DC., *Cir. kamtschaticum* Ledeb. ex DC., *Cir. setosum* (Willd.) M. Bieb., and two exotic species (*Cir. vulgare* (Savi) Tenore and *Cir. arvense* (L.) Scopoli) also occurred sporadically (Table 1). Two or more thistle species often co-occurred at a single site.

The beetle *Cassida rubiginosa* was observed at 74 of 97 sites (76.3% of the sites investigated) (Fig. 1) on seven thistle species other than the two exotic ones (Table 1). In contrast, the target species, *C. vibex*, was observed at only four sites in two remote areas (Fig. 1): one site at Nanae, Kameda (42°00'N, 140°38'E), and three sites at



Shiriuchi. *Cassida vibex* occurred on *Cir. alpicola*, with the exception of subsidiary utilization of *Cir. grayanum* at one site at Shiriuchi (Table 1). The population density of *C. vibex* was very low, and we observed only one to several adult beetles at three of the sites. At one site at Shiriuchi, *C. vibex* was relatively abundant on *Cir. alpicola* and also used *Cir. grayanum*; at this site in 2004, three adult *C. vibex* and an ootheca of this species were observed on *Cir. grayanum*, although no larvae developing on this thistle were found. At all four sites, *C. vibex* co-occurred with *C. rubiginosa* (Fig. 1) on the same host plant species and sometimes even on the same host plant individuals.

### **Adult feeding preference**

Neither adult female nor male *C. vibex* showed a significant feeding preference for particular thistle species in any of the three combinations of thistles tested ( $P > 0.05$  in all cases) (Fig. 2).

### **Larval performance**

Eclosion rates were moderate in 2003 (the highest was 64.0% on *Cir. aomorense*) (Table 2a) and were lower in 2005 than that in 2003 (ranging from 14.0% on *Cir. grayanum* to 38.0% on *Cir. alpicola*) (Table 2b). A logistic regression analysis detected significant effects of food (d.f. = 3,  $\chi^2 = 12.89$ ,  $P = 0.0049$ ) and year (d.f. = 1,  $\chi^2 = 26.55$ ,  $P < 0.0001$ ), but no significant effect for the food  $\times$  year interaction (d.f. = 3,  $\chi^2 = 3.07$ ,  $P = 0.38$ ). Therefore, pair-wise comparisons among food conditions were conducted for the pooled data from the two years. The overall eclosion rate was highest on *Cir. alpicola* (49.0%), somewhat less on *Cir. aomorense* (47.0%) and *Cir. yezoense* (43.0%), and lowest on *Cir. grayanum* (28.0%). The difference in eclosion rates was significant only for *Cir. alpicola* vs *Cir. grayanum* (d.f. = 1,  $G = 9.33$ ,  $P = 0.013$ ) and *Cir. aomorense* vs *Cir. grayanum* (d.f. = 1,  $G = 7.70$ ,  $P = 0.033$ ;  $G$ -test for multiple comparisons) (Sokal & Rohlf 1995).

Mixed-model ANOVAs detected no significant effect of food on either

developmental period or body size (Table 3). For developmental period, the year factor showed a significant effect, reflecting a tendency for faster growth in 2005. A significant effect of the food  $\times$  year interaction indicated inconsistency between years in the rank order of suitability among thistle species affecting this trait (Tables 2, 3). For pronotum width,  $r^2$  (the overall fit to the model) in the ANOVA was somewhat small, suggesting that body size varied unpredictably, regardless of the other factors included in our analysis, although a significant effect of sex reflected the larger size of females (Tables 2, 3).

## DISCUSSION

Our field survey detected *C. vibex* in two remote areas (Nanae and Shiriuchi) on the Oshima Peninsula in southwestern Hokkaido (Fig. 1). This is the first published record of *C. vibex* for Hokkaido, and Nanae can be tentatively regarded as the northern limit of the range of this beetle in the Japanese Archipelago. We did not find this beetle on wild thistles during intensive field studies of thistle-feeding *Henosepilachna* ladybird beetles in more northern areas ranging from the vicinity of Sapporo (43°04'N, 141°21'E) to Asahikawa (43°46'N, 142°22'E) and as far north as Otoineppu (44°43'N, 142°16'E) (Fujiyama & Katakura 1997; Fujiyama *et al.* 2003; N. Fujiyama, unpublished data, 2010). This fact suggests that *C. vibex* is restricted to the Oshima Peninsula on Hokkaido.

The fauna and flora of the Oshima Peninsula are somewhat different from other regions of Hokkaido (e.g. Tatewaki 1958; Ishikawa 1968; Munakata & Yamane 1970; Higashi *et al.* 1993; Uemura 1994; Katakura 1997; Inoko 1999). The peninsula comprises the southwestern part of Hokkaido but is climatically and vegetationally rather similar to northern Honshu, separated from Hokkaido by Tsugaru Strait. The Oshima Peninsula harbors both northern elements common to other regions of Hokkaido and southern elements that have extended their ranges from the south, in

addition to species widespread in Japan. For example, a herbivorous ladybird beetle *Henosepilachna niponica* (Lewis) and a lycaenid butterfly *Sibataniozephyrus fujisanus* (Matsumura) are typical representatives of the southern elements (Katakura 1997; Inoko 1999; Shirouzu 2006). *Cassida vibex* can also be regarded as one of the southern elements, though distributional records on Hokkaido are scanty.

It is noteworthy that *C. vibex* was found at only four of 97 study sites, mainly on *Cir. alpicola* among several thistle species available, and occurred in sometimes very low density. The low density coupled with a restricted distribution in area and on host plants contrasts greatly with the frequent occurrence of the congeneric species *C. rubiginosa* on various thistle species (Fig. 1, Table 1). We initially expected that the distribution of *C. vibex* on the Oshima Peninsula would be determined by the distribution of *Cir. alpicola*, like as the case of the thistle-feeding ladybird beetle *H. niponica* (Koizumi *et al.* 1999); however, *C. vibex* was found at only four of the 29 sites where we detected *Cir. alpicola* (in contrast, the congener *C. rubiginosa* was found at 23 of the 29 sites) (Table 1). In addition, the laboratory experiments showed no preference by *C. vibex* for *Cir. alpicola* over three other thistle species (*Cir. grayanum*, *Cir. yezoense*, and *Cir. aomorense*) also common on the Oshima Peninsula (Fig. 2), and *Cir. alpicola*, *Cir. yezoense*, and *Cir. aomorense* were nearly equally suitable for the growth of *C. vibex* larvae, though the survival rate on *Cir. grayanum* was lower (Table 2).

One of the additional factors determining the distributional pattern of *C. vibex* on the Oshima Peninsula would be an effect of host plant abundance in each habitat. The four thistle species common on the Oshima Peninsula differed in degree of aggregation, although we did not gather quantitative data to describe these differences. *Cirsium grayanum* and *Cir. alpicola* were often clumped, with many individual plants in a narrow area, whereas *Cir. yezoense* and *Cir. aomorense* did not form large clumps, and individual plants were rather scattered within the respective habitats. On the Oshima Peninsula, *Cir. grayanum* is most abundant in various habitats from forest margins to

grasslands (Table 1; Koizumi *et al.* 1997), but it is not the best host plant for the growth of *C. vibex* larvae (Table 2). The other three thistles are equally suitable or nearly so, but among them, *Cir. aomorense* and *Cir. yezoense* are scattered although not rare. If the persistence of *C. vibex* requires a certain level of host plant abundance, only large and dense patches of *Cir. alpicola* satisfy the requirements of *C. vibex* on the Oshima Peninsula. Indeed, the density of *Cir. alpicola* was high in the habitats where we found *C. vibex*. We postulate that the persistence of *C. vibex* is possible only where a substantial amount of suitable host plants is available. Of course, this is a tentative explanation for the scarcity and biased distribution of *C. vibex* on the Oshima Peninsula. Other additional possible factors such as interactions with other herbivores or natural enemies, and climate conditions near the tentative northern limit of the distribution of *C. vibex* in Japan, should be tested by future studies.

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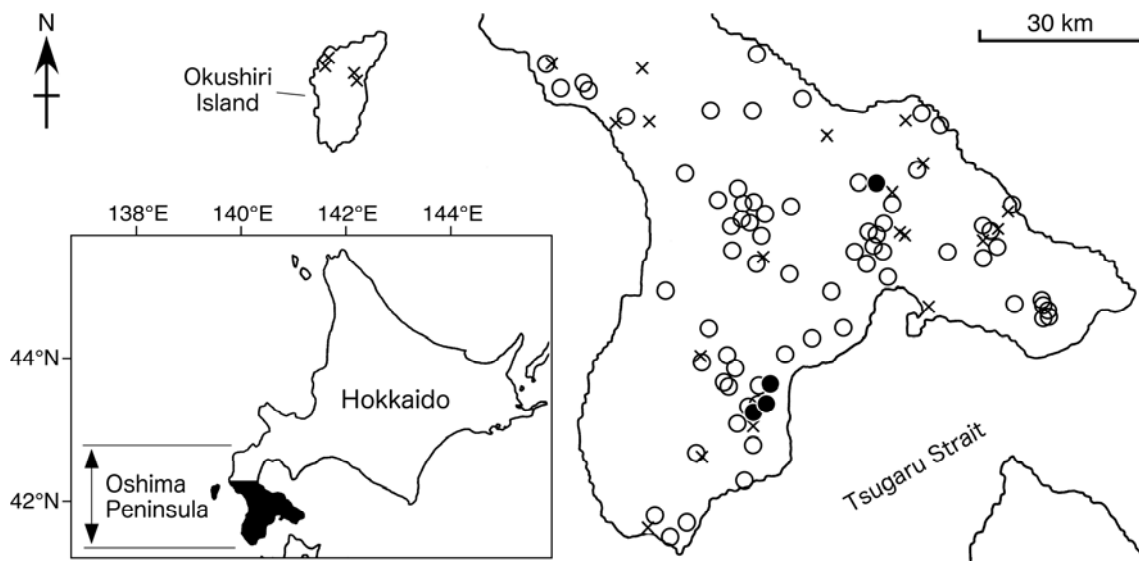
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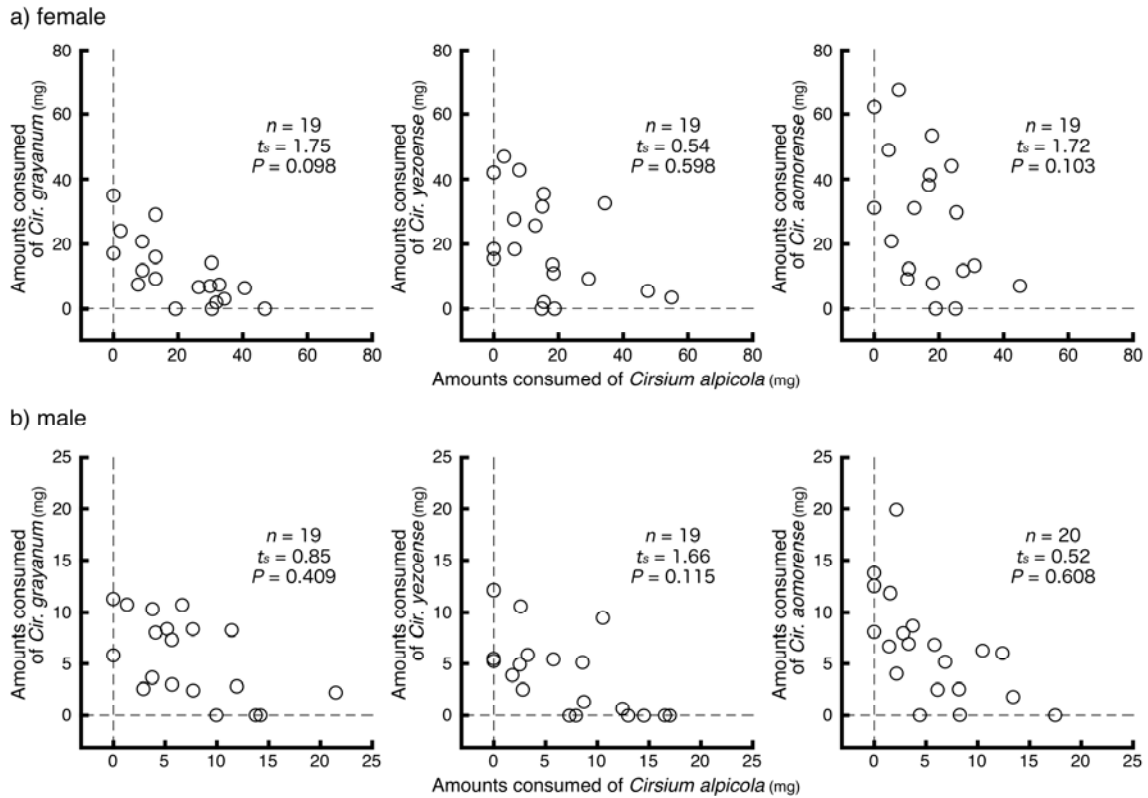
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**Figure 1** Distributions of the two thistle-feeding tortoise beetles, *Cassida vibex* and *C. rubiginosa*, in the southern part of the Oshima Peninsula, Hokkaido, northern Japan, from data obtained from 2003 through 2006. Co-occurrence of the two beetle species (●); only *C. rubiginosa* detected (○); neither *Cassida* species detected (×).





**Figure 2** Choices made by posthibernating adults of the tortoise beetle *Cassida vibex* for *Cirsium alpicola* and the other three *Cirsium* species. Each dot represents a Leaf amount consumed by a beetle over two successive days is represented by ○. Statistically significant differences ( $P \leq 0.05$ ) indicate a preference for either plant, as determined by larger amounts consumed (analyzed by Student's *t*-test for paired comparisons).

**Table 1** Number of sites where each thistle species was detected, and occurrence of the two thistle-feeding tortoise beetles, *Cassida vibex* and *C. rubiginosa*, on thistle species in southwestern Hokkaido, northern Japan (investigated from 2003 through 2006)

Thistle species	No. of sites	% occurrence of beetles (No. of sites)	
		<i>C. vibex</i>	<i>C. rubiginosa</i>
<i>Cirsium alpicola</i>	29	13.8 (4)	79.3 (23)
<i>Cir. grayanum</i>	70	1.4 (1)	80.0 (56)
<i>Cir. yezoense</i>	22	0.0 (0)	27.3 (6)
<i>Cir. aomorense</i>	26	0.0 (0)	34.6 (9)
<i>Cir. pendulum</i>	6	0.0 (0)	50.0 (3)
<i>Cir. kamtschaticum</i>	3	0.0 (0)	33.3 (1)
<i>Cir. setosum</i>	4	0.0 (0)	50.0 (2)
<i>Cir. vulgare</i>	7	0.0 (0)	0.0 (0)
<i>Cir. arvense</i>	1	0.0 (0)	0.0 (0)
Total <sup>†</sup>	97	4.1 (4)	76.3 (74)

<sup>†</sup>More than one thistle species occurred at some sites.

**Table 2** Larval developmental performance (mean  $\pm$  SE (*n*)) of the tortoise beetle *Cassida vibex* on four thistle species (*Cirsium* spp.)

a) 2003

Food	Eclosion rate in %	Developmental period in days	Pronotum width in mm	
			Female	Male
<i>Cir. alpicola</i>	60.0	39.2 $\pm$ 0.36 (30)	4.6 $\pm$ 0.05 (12)	4.5 $\pm$ 0.03 (18)
<i>Cir. grayanum</i>	42.0	38.2 $\pm$ 0.60 (21)	4.6 $\pm$ 0.05 (10)	4.4 $\pm$ 0.03 (11)
<i>Cir. yezoense</i>	50.0	39.6 $\pm$ 0.45 (25)	4.7 $\pm$ 0.02 (13)	4.5 $\pm$ 0.02 (12)
<i>Cir. aomorense</i>	64.0	38.1 $\pm$ 0.31 (32)	4.7 $\pm$ 0.03 (12)	4.5 $\pm$ 0.03 (20)

b) 2005

Food	Eclosion rate in %	Developmental period in days	Pronotum width in mm	
			Female	Male
<i>Cir. alpicola</i>	38.0	34.9 $\pm$ 0.39 (19)	4.5 $\pm$ 0.08 (6)	4.5 $\pm$ 0.04 (13)
<i>Cir. grayanum</i>	14.0	36.6 $\pm$ 0.72 (7)	4.4 $\pm$ 0.35 (2)	4.4 $\pm$ 0.04 (5)
<i>Cir. yezoense</i>	36.0	34.4 $\pm$ 0.39 (18)	4.7 $\pm$ 0.03 (9)	4.5 $\pm$ 0.02 (9)
<i>Cir. aomorense</i>	30.0	33.0 $\pm$ 0.22 (15)	4.7 $\pm$ 0.03 (7)	4.5 $\pm$ 0.03 (8)

**Table 3** Mixed-model ANOVAs for developmental period and pronotum width of the tortoise beetle *Cassida vibex* reared on four thistle species (*Cirsium* spp.) in 2003 and 2005

Source	Developmental period			Pronotum width		
	N <sub>d.f.</sub>	D <sub>d.f.</sub>	<i>F</i>	N <sub>d.f.</sub>	D <sub>d.f.</sub>	<i>F</i>
Food	3	3.00	1.34	3	3.02	5.74
Year	1	3.08	30.09*	1	3.48	0.86
Food × year	3	158	5.76***	3	158	1.00
Sex	1	158	3.34	1	158	52.55***
<i>r</i> <sup>2</sup>	0.590			0.302		

Analyzed after logarithmic transformation. N<sub>d.f.</sub> and D<sub>d.f.</sub> indicate the degrees of freedom of the mean squares used as the numerator and denominator, respectively, in calculating *F*. The food × year interaction was used as the error term to test for the effects of food and year. *r*<sup>2</sup> indicates the overall fit of the model.

\*  $0.01 < P \leq 0.05$ , \*\*\*  $P \leq 0.001$ .