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ORIGINAL ARTICLE

Nobuhiko Suzuki · Toru Ide

The foraging behaviors of larvae of the ladybird beetle, *Coccinella septempunctata* L., (Coleoptera: Coccinellidae) towards ant-tended and non-ant-tended aphids

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Abstract The foraging behaviors of larvae of the ladybird, *Coccinella septempunctata* L., towards both the ant-tended aphid, *Aphis craccivora* Koch, and the non-ant-tended aphid, *Acyrthosiphon pisum* Harris, were investigated in the field and in laboratory experiments. Although there were no differences in the development and growth of the ladybird larvae that preyed on either *Ac. pisum* or *Ap. craccivora*, the foraging efficiency of the ladybird larvae that preyed on *Ap. craccivora* was higher than that of the ladybird larvae that preyed on *Ac. pisum* in the absence of ants. This result was explained by the fact that the number of *Ac. pisum* that escaped by dropping off the plant was conspicuously larger than the number of *Ap. craccivora* that escaped in this fashion and derived from the non-ant-attendance associated with *Ac. pisum*. In the laboratory experiments, fewer ladybird larvae climbed onto a plant with *Ap. craccivora* in the presence of ants than onto a plant with *Ac. pisum* in the absence of ants. The ladybird larvae did not switch from foraging for *Ap. craccivora* to foraging for *Ac. pisum*, even after suffering attacks by ants on a plant with *Ap. craccivora*, and it would appear that ladybird larvae are unable to remember where they have previously been attacked by ants. These results could explain why the ladybird larvae in the field more frequently visited *Vicia angustifolia* plants with *Ap. craccivora* than those with *Ac. pisum* and made more visits when ants were absent than when they were present.

Keywords *Acyrthosiphon pisum* · Ant-attendance · *Aphis craccivora* · Foraging efficiency · Prey preference

N. Suzuki (✉) · T. Ide
Department of Applied Biological Sciences,
Faculty of Agriculture, Saga University,
Honjo 1, Saga 840-8502, Japan
E-mail: suzukin@cc.saga-u.ac.jp
Tel.: +81-952-288792
Fax: +81-952-288792

Introduction

Numerous studies on the prey preferences of aphidophagous ladybird larvae have been carried out (Blackman 1967; Omkar et al. 1997; Kalushkov 1998; Michaud 2000; Nielsen et al. 2002; Omkar and Srivastava 2003; Kalushkov and Hodek 2004; Omkar and James 2004). As is the case in many predatory insect species, the larvae of aphidophagous ladybird species frequently prey on a wide range of aphid species (Hodek and Honek 1996; Dixon 2000). However, the aphids of the various species are not equally suitable as a food resource of ladybird larvae, and ladybird larvae thus frequently prefer to feed on a certain prey.

The searching behaviors of aphidophagous ladybird larvae have attracted the interest of numerous investigators (Banks 1957; Kehat 1968; Murdoch and Marks 1973; Storch 1976; Marks 1977; Hunter 1978; Carter and Dixon 1982, 1984; Carter et al. 1984; Murakami and Tsubaki 1984; Hajek and Dahlsten 1987; Ferran and Dixon 1993; Clark and Messina 1998; Biesinger and Haefner 2005). Such behavior has been thought to be nearly random, with the larvae unable to detect prey before actual contact (Banks 1954; Kehat 1968). However, the searching that is conducted after preying is an intensive area-restrictive search in which the ladybird larvae move slowly and turn more frequently (Banks 1957; Marks 1977; Murakami and Tsubaki 1984; Biesinger and Haefner 2005). Furthermore, it is likely that, as seen in ladybird adults, the larvae of some ladybird species also use chemical cues in searching for prey (Stubbs 1980; Jamal and Brown 2001).

Most of the studies that have been conducted on the prey preference and foraging behaviors of aphidophagous ladybird larvae have focused on foraging for non-ant-tended aphids. This may be due to the fact that ant-tended aphids are protected by ants and, therefore, ladybird larvae are frequently excluded by ants from aphid colonies and that they are less able to feed on ant-tended aphids. In many cases, however, the mutualism between aphids and ants is facultative, and ants do not always tend aphids, particularly in temperate regions (Bristow 1991; Stadler and Dixon 1999; 2005). Therefore, if ants do not guard the aphids, the ladybird larvae can easily feed on ant-tended aphids. Non-ant-tended aphids, however, have developed various mechanisms by which they avoid predation and/or parasitism (Hight et al. 1972; Roitberg and Myers 1978; Arakaki 1989; Völkl and Stadler 1996; Dixon 1998). For example, many non-ant-tended aphids secrete an alarm pheromone when captured by a predator, and adjacent aphids that receive the alarm pheromone then drop off the host plants or escape by walking (Kislow and Edwards 1972; Bower et al. 1972; Edwards et al. 1973; Nault et al. 1973; Montgomery and Nault 1977; Roitberg and Myer 1978; Dill et al. 1990; Chau and Mackauer 1997; Dixon 1998; Losey and Denno 1998).

Despite conspicuous differences in the manners of predator avoidance between ant-tended and non-ant-tended aphids, the prey preference and foraging behaviors of the aphidophagous ladybird larvae have not yet been studied in association with ant-attendance on aphids. Hence, we addressed the question how aphidophagous ladybird larvae forage for ant-tended and non-ant-tended aphids in the presence and absence of ants, respectively.

The larvae of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) frequently feed on *Aphis craccivora* Koch and *Acyrthosiphon pisum* Harris (Homoptera: Aphididae). In western Japan, the aphids of these two species frequently colonize on leguminous plants, such as the broad bean *Vicia faba* L. and the vetch *Vicia angustifolia* L. (Leguminosae) (Suzuki et al. 2004). *Ap. craccivora* is an ant-tended aphid that can gain the protective service of ants attracted to its honeydew (Katayama and Suzuki 2002; 2003). The mutualism between *Ap. craccivora* and ants is facultative, and the aphids are therefore not always tended by ants. Consequently, *Ap. craccivora* would be expected to show less escape behavior from predators when *Ap. craccivora* is tended by ants. On the other hand, *Ac. pisum,* which is not tended by ants, drops off host plants to escape predation (Nault et al. 1973; Losey and Denno 1998).

In this study, we investigated the number of *C. septempunctata* larvae that visited plants of *V. angustifolia* colonized by *Ap. craccivora* or *Ac. pisum* in the field in an attempt to elucidate the foraging strategy of ladybird larvae for both ant-tended and non-ant-tended aphids. In addition, the quality of *Ap. craccivora* and *Ac. pisum* as food resources and the foraging behaviors of *C. septempunctata* larvae towards *Ap. craccivora* and *Ac. pisum* in the presence and absence of ant guards were examined in laboratory experiments. The foraging strategy of ladybird larvae is discussed in relation to ant-attendance.

Materials and methods

Study species

Aphis craccivora and *Acyrthosiphon pisum* were collected from *Vicia angustifolia* plants on the campus of Saga University, Saga City, western Japan (33°14′N, 130°18′E). Aphids of each species were separately reared on seedlings of *V. faba* maintained in plastic cages (length: 32 cm, width: 25 cm, height: 25 cm) fitted with a nylon mesh over the top, at 20°C under a 12/12-h (light/dark) photoperiod.

Adult females of *C. septempunctata* were collected on the campus of Saga University. These were singly reared in petri dishes (diameter: 9 cm, depth: 1.5 cm) containing approximately equal amounts of *Ap. craccivora* and *Ac. pisum* supplied daily as food. Eggs of *C. septempunctata* were obtained from these adults. Hatched larvae were reared singly in petri dishes (diameter: 9 cm, depth: 1.5 cm) in the laboratory at 20°C under a 12/12-h (light/dark) photoperiod, with approximately equal amounts of *Ap. craccivora* and *Ac. pisum* supplied daily as food.

Workers of *Lasius japonicus* Santschi (Hymenoptera: Formicidae) are medium-sized ants (body length: about 4 mm). They prefer sugars and frequently feed on honeydew and are aggressive when other insects invade their colony and feeding sites (Itioka and Inoue 1999). Colonies of *L. japonicus* were collected in Kubota-Cho, Saga and then transferred to several test tubes (diameter: 1.2 cm; length: 12 cm). Three hundred workers were placed in each tube. The bottom of each tube was packed with wet cotton wool (depth: about 3 cm) to maintain a suitable level of humidity. The tube was covered with aluminum foil to maintain darkness as an ant nest. Each test tube was connected to a vinyl chloride tube 6 mm in inner diameter and 10 cm long to form an entrance. The ants were fed a 10% sucrose solution from a test tube (diameter: 1.2 cm; length: 12 cm) plugged with cotton wool. The ants in each nest were starved for 3 days before the commencement of the experiments in order to increase their hunger level and stimulate searching behavior.

Field census

The field census was carried out between April and May 2005 on *V. angustifolia* plants growing in grasslands located along the riverside of Kase River, Kubota-Cho, Saga. Many colonies of *Ap. craccivora* and *Ac. pisum* were found on the *V. angustifolia* plants. During the field census, we carried out 783 and 753 observations on 261 *Ap. craccivora* colonies and 251 *Ac. pisum* colonies, respectively. We counted the numbers of aphids (colony size), ladybird larvae, and ants visiting *V. angustifolia* plants with *Ap. craccivora* and *Ac. pisum* colonies.

Experiment 1. The quality of *Ap. craccivora* and *Ac. pisum* as food resources

One-day-old hatchlings of the ladybird were reared individually on wet paper (2 × 2 cm) in a glass petri dish (diameter: 5 cm in diameter, depth: 1.5 cm) at 20°C under a 12/12-h (light/dark) photoperiod. Sufficient amounts of *Ap. craccivora* or *Ac. pisum* were supplied as a food each day. The molting of larvae and adult eclosion were checked each day, and the duration of development from the first instar to the adult and the wet weight of the eclosed adults were measured. The experiments were replicated ten times for feeding on each aphid species.

Experiment 2. The foraging efficiency for *Ap. craccivora* in the absence of ants, for *Ac. pisum* in the absence of ants, and for *Ap. craccivora* in the presence of ants

Seedlings of *V. faba* were grown in polyethylene pots (diameter: 9 cm, depth: 6.5 cm) at 20°C under a 12/12-h (light/dark) photoperiod. Plastic pots (diameter: 10 cm, depth: 4.5 cm) were filled with water and covered with a petri dish lid with a 1.5-cm hole in the center. The stem of each *V. faba* seedling with three leaf nodes (each leaf node with two leaves) was pushed through the hole. The base of the seedling was covered with wet cotton wool to prevent it from drying and to stabilize the stem.

One hundred *Ap. craccivora* adults or 100 *Ac. pisum* young nymphs that had a body size similar to *Ap. craccivora* adults were released onto each *V. faba* plant. The experimental apparatus (diameter: 53 cm, depth: 16.5 cm) was covered with plaster to a depth of 5 cm, with three holes (positions A, B, C) in which plastic pots with plants were set. The inner sides of the apparatus wall were coated with talc powder to prevent ladybird larvae from escaping. The plant with aphids was set at position A on the experimental apparatus (Fig. 1a).

We used fourth instar ladybird larvae in the experiments. The larvae were fed only water for 6 h prior to the commencement of experiments in order to increase their hunger level and stimulate searching behavior.

The experiment was carried out at 25°C under a fluorescent light (27 W) in the laboratory. One fourth instar larva was released into the experimental apparatus. In addition, in the experiment for foraging for *Ap. craccivora* in the presence of ants, one ant nest was set in the experimental apparatus just after the ladybird larva climbed onto the plant. The observation was carried out from the time that the ladybird larva climbed onto the plant to the time when it got off the plant. The resident time on the plant, foraging behaviors of the ladybird larva, comprising attacks to the aphids and predation, and the escape behaviors of the aphids were examined. Aphids that dropped off the plant were removed from the experimental apparatus. The experiments were replicated 20, 20, and 100 times for foraging for *Ap. craccivora* in the absence of ants, *Ac. pisum* in the absence of ants and *Ap. craccivora* in the presence of ants, respectively. The experimental apparatus was cleaned by washing with water between replicates. The same plants were used only once in each replicate.

Experiment 3. Choice of plants with *Ap. craccivora* in the presence of ants or with *Ac. pisum* in the absence of ants

The experimental apparatus (length: 19 cm, width: 25 cm, height: 9 cm) was covered with plaster to a depth of 5 cm with two holes into which the plastic pots with plants were set. The inner sides of the apparatus wall were coated with a talc powder to prevent a ladybird larva from escaping.

One hundred aphids of either *Ap. craccivora* or *Ac. pisum* were released on each *V. faba* plant. After 24 h, a plant with *Ap. craccivora* was set in a hole on the experimental apparatus. One ant nest was set in the experimental apparatus, and the ants were allowed to forage on the plant. At 90 min after the setting of the ant nest, when ant activity had stabilized, another plant with *Ac. pisum* was set in another hole on the experimental apparatus. Ants that visited the plant with *Ac. pisum* were removed.

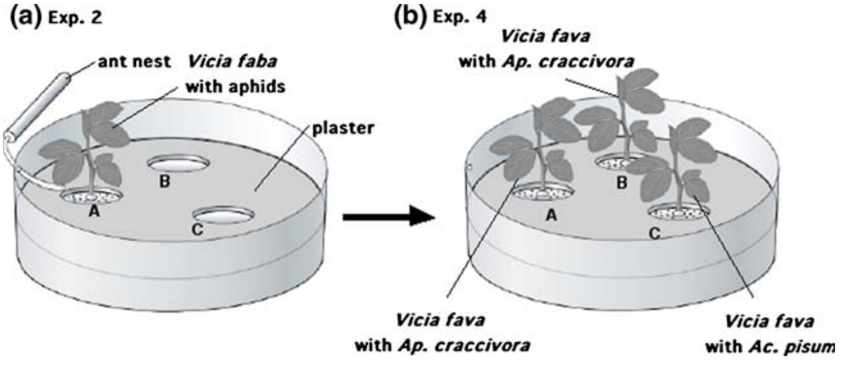

Fig. 1 The experimental apparatus used in the experiments on the foraging efficiency of *Coccinella septempunctata* larvae for *Aphis craccivora* in the absence of ants, *Acyrthosiphon pisum* in the absence of ants and *Ap. craccivora* in the presence of ants (*Exp. 2*) (**a**), and the choice of plants with *Ap. craccivora* or *Ac. pisum* after experiencing attacks by ants on the plant with *Ap. craccivora* (*Exp. 4*) (**b**)

The experiment was carried out at 25°C under a fluorescent light (27 W) in a laboratory. One fourth instar larva was released in the center of the experimental apparatus. The experiments were replicated 30 times. The experimental apparatus was cleaned by washing with water between replicates. Plants were used only once in each replicate. We examined the number of larvae arriving at petri dish lids under the plants with *Ap. craccivora* in the presence of ants and at those under the plants with *Ac. pisum* in the absence of ants, the number of visits to the petri dish lids, the resident time of the ladybird larvae on petri dish lids and the number of larvae climbing onto the plants with *Ap. craccivora* in the presence of ants and with *Ac. pisum* in the absence of ants. The experiment was terminated when the larva climbed onto a plant. If a larva did not climb onto a plant for 30 min, we no longer continued the observation.

Experiment 4. Choice of plants with *Ap. craccivora* or *Ac. pisum* after experiencing attacks by ants on the plant with *Ap. craccivora*

Just after Experiment 2 was completed and the larva had left the plant and were wandering on the floor of the experimental apparatus, the plant with *Ap. craccivora* at position A, ants wandering around the experimental apparatus and the ant nest were all removed from the experimental apparatus. Two new plants with *Ap. craccivora* (plants A and B) were set at positions A and B, and another plant with *Ac. pisum* (plant C) was set at position C (Fig. 1b).

The experiment was carried out at 25°C under a fluorescent light (27 W) in a laboratory. We examined which three plants the larvae that had been attacked by ants at position A chose. The numbers of larvae arriving at the petri dish lids under the plant and climbing onto the plants were examined. The experiment was terminated when the larva climbed onto a plant. If the larva did not climb onto a plant for 30 min, we no longer pursued the observation. The experiments were replicated 50 times. The experimental apparatus was cleaned by washing with water between replicates. The same plants used only once in each replicate.

Statistical analysis

In the field census, the numbers of ladybird larvae and ants visiting *V. angustifolia* plants with *Ap. craccivora* and *Ac. pisum* in the presence and absence of ants were compared by two-way ANOVA and Student's *t*-test with Bonferroni adjustment for multiple tests. Comparisons of the quality of the two aphid species as food resources in Experiment 1 and those on the number of visits and the resident time on petri dish lids under the plants in Experiment 3 were analyzed by the Mann–Whitney *U*-test. The comparisons in foraging behaviors of the ladybird larvae and anti-predatory behaviors of aphids in Experiment 2 were analyzed by Kruskal–Wallis test and the Mann–Whitney *U*-test with Bonferroni adjustment for multiple tests. The number of larvae arriving at petri dish lids and climbing onto the plants in Experiment 3 were compared by the binomial test. The number of larvae arriving at petri dish lids and climbing onto the plants in Experiment 4 were analyzed by χ^2-test for independence.

Results

Field census

Ants visited *V. angustifolia* plants with ant-tended *Ap. craccivora* and even with non-ant-tended *Ac. pisum* because *V. angustifolia* plants bore extrafloral nectaries. Although four ant species, *Lasius japonicus* Santschi, *Pristomyrmex pungens* Mayr, *Tetramorium caespitum* Linnaeus and *Formica japonica* Motschulsky were found on *V. angustifolia* plants, workers of three of these species, with the exception of the workers of *L. japonicus*, seldom visited *V. angustifolia* plants.

The colony size of *Ap. craccivora* was larger than that of *Ac. pisum*, both in the presence and absence of ants (in the presence of ants: *Ap. craccivora*, $n = 324$, mean ± SE: 212.43 ± 7.80, *Ac. pisum*, $n = 169$, 138.36 ± 11.01, $t = -5.52$, $P < 0.0001$; in the absence of ants: *Ap. craccivora*, $n = 459$, 193.93 ± 7.06, *Ac. pisum*, $n = 584$, 113.61 ± 4.28, $t = -10.16$, $P < 0.0001$). In the presence of ants, a larger number of ants visited *V. angustifolia* plants with *Ap. craccivora* than those with *Ac. pisum* (*Ap. craccivora*: $n = 324$, mean ± SE: 5.73 ± 0.23, *Ac. pisum*: $n = 169$, 2.60 ± 0.18, $t = -9.26$, $P < 0.0001$).

The number of ladybird larvae that visited *V. angustifolia* plants differed between *Ap. craccivora* and *Ac. pisum* and also differed in the presence and absence of ants (two-way ANOVA, aphid: $F = 32.765$, $P < 0.0001$; ant: $F = 32.559$, $P < 0.0001$; aphid × ant: $F = 8.422$, $P = 0.0038$; Fig. 2). Both in the presence and absence of ants, the number of ladybird larvae that visited *V. angustifolia* plants with *Ap. craccivora* was larger than the number that visited *V. angustifolia* plants with *Ac. pisum* (in the presence of ants: $t = -2.93$, $P = 0.0035$; in the absence of ants: $t = -6.83$, $P < 0.0001$). Both on *V. angustifolia* plants with *Ap. craccivora* and those with *Ac. pisum*, the number of ladybird larvae was larger in the absence of ants than in the presence of ants (*Ap. craccivora*: $t = -5.52$, $P < 0.0001$; *Ac. pisum*: $t = -2.64$, $P = 0.0088$).

The quality of *Ap. craccivora* and *Ac. pisum* as food resources

The duration of development from the first instar to the adult did not differ between the ladybird larvae that fed on *Ap. craccivora* and those that fed on *Ac. pisum* (*Ap. craccivora*: $n = 10$, mean ± SE: 23.50 ± 0.17 days; *Ac.*

pisum: $n = 10$, 23.60 ± 0.22 days, $z = -0.70$, $P = 0.51$). The body weight of the eclosed adults that developed by feeding on *Ap. craccivora* and *Ac. pisum* also did not differ (*Ap. craccivora*: $n = 10$, mean ± SE: 39.24 ± 1.08 mg, *Ac. pisum*: $n = 10$, 37.85 ± 0.89 mg, $z = -0.83$, $P = 0.41$).

The foraging efficiency for *Ap. craccivora* in the absence of ants, for *Ac. pisum* in the absence of ants, and for *Ap. craccivora* in the presence of ants

The foraging behaviors of the ladybird larvae and anti-predatory behaviors of aphids in the experiments for foraging for *Ap. craccivora* in the absence of ants, for *Ac. pisum* in the absence of ants, and for *Ap. craccivora* in the presence of ants are shown in Table 1. The number of aphids attacked by the ladybird larvae per hour did not differ between *Ap. craccivora* in the absence of ants and *Ac. pisum* in the absence of ants, but it was extremely low on *Ap. craccivora* in the presence of ants. The predation success rate (number of aphids eaten/number of aphids attacked) was higher on *Ap. craccivora* in the absence of ants than on *Ac. pisum* in the absence of ants, but it did not differ between *Ap. craccivora* in the presence and in the absence of ants. The number of aphids eaten by a ladybird larva per hour was higher on *Ap. craccivora* in the absence of ants than on *Ac. pisum* in the absence of ants, and was extremely low on *Ap. craccivora* in the presence of ants.

The number of aphids that escaped from ladybird larvae by dropping from the plant (per hour) was much higher on *Ac. pisum* in the absence of ants than on *Ap. craccivora* in the presence and absence of ants, respectively. However, the number of aphids that escaped from the ladybird larvae by walking (per hour) was conspicuously higher on *Ap. craccivora* in the absence of ants and was the lowest on *Ac. pisum* in the absence of ants.

The resident time of the ladybird larvae on the plants was longest on *Ap. craccivora* in the absence of ants and shortest on *Ap. craccivora* in the presence of ants.

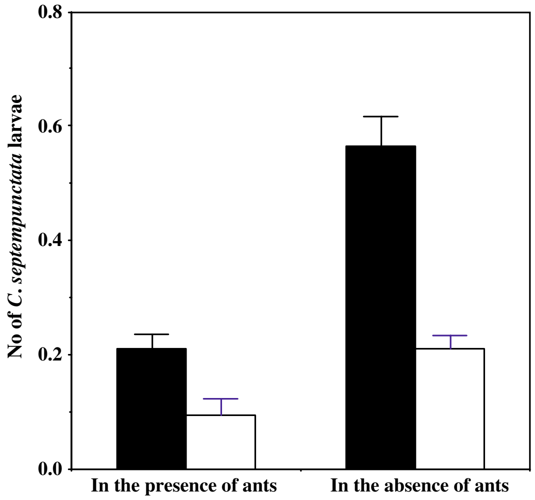

Fig. 2 The number of ladybird larvae that visited *V. angustifolia* plants with *Ap. craccivora* (*solid column*) and those with *Ac. pisum* (*open column*) in the presence and absence of ants in the field. *Bars*: standard error

Choice of plants with *Ap. craccivora* in the presence of ants or with *Ac. pisum* in the absence of ants

The number of ladybird larvae arriving at petri dish lids under each plant did not differ between the petri dish lids under the plant with *Ap. craccivora* in the presence of ants and with *Ac. pisum* in the absence of ants (binomial test: $P = 0.43$; Fig. 3a). Although the number of visits onto the petri dish lid did not differ between the petri dish lid under the plant with *Ap. craccivora* in the presence of ants and that under the plant with *Ac. pisum* in the absence of ants (*Ap. craccivora*: $n = 30$, mean ± SE: 2.20 ± 0.39; *Ac. pisum*: $n = 30$, 1.73 ± 0.21, $z = -0.45$, $P = 0.65$), the resident time of the ladybird larvae on the petri dish lid was longer on the petri dish

Table 1 The foraging behaviors of *Coccinella septempunctata* larvae and anti-predatory behaviors of aphids in the experiments in terms of foraging on *Aphis craccivora* in the absence of ants, on *Acyrthosiphon pisum* in the absence of ants and on *Ap. craccivora* in the presence of ants

	In the absence of ants				In the presence of ants		P^a
	Ap. craccivora		*Ac. pisum*		*Ap. craccivora*		
	n	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE	
Number of aphids attacked per hour	20	14.09 ± 0.68 a	20	14.97 ± 1.98 a	100	5.70 ± 0.52 b	< 0.0001
Predation success rate	20	0.98 ± 0.01 a	20	0.74 ± 0.02 b	81	0.81 ± 0.03 ab	0.0011
Number of aphids eaten per hour	20	13.74 ± 0.67 a	20	10.98 ± 1.42 b	100	4.22 ± 0.34 c	< 0.0001
Time needed to feed on an aphid (s)	20	96.90 ± 2.84 a	20	93.55 ± 3.67 a	79	121.98 ± 6.98 b	0.0027
Number of aphids escaped by dropping per hour	20	9.36 ± 1.48 a	20	59.05 ± 7.75 b	100	6.50 ± 0.95 a	< 0.0001
Number of aphids escaped by walking per hour	20	12.86 ± 2.14 a	20	2.26 ± 0.61 b	100	4.18 ± 0.59 c	< 0.0001
Resident time on a plant (s)	20	7942.80 ± 613.83 a	20	3500.60 ± 429.89 b	100	1438.20 ± 107.20 c	< 0.0001

[a] Kruskal–Wallis test

Values followed by different letters indicate significant differences (Mann–Whitney *U*-test with Bonferroni adjustment for multiple tests, $P < 0.0167$)

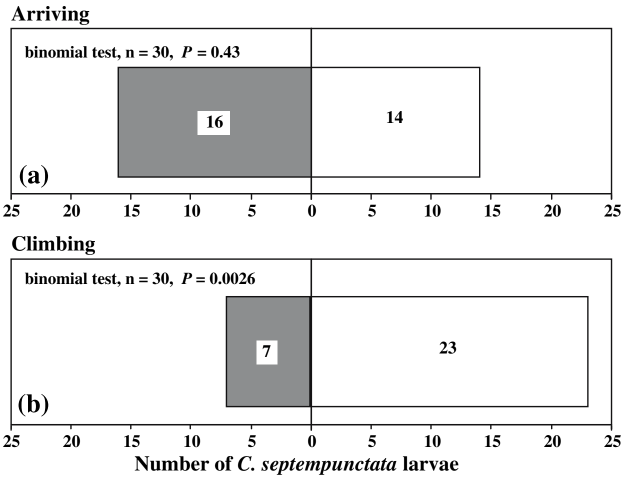

Fig. 3 Choice of plants with *Ap. craccivora* in the presence of ants or with *Ac. pisum* in the absence of ants by *C. septempunctata* larvae. **a** The number of *C. septempunctata* larvae arriving at petri dish lids under the plant with *Ap. craccivora* in the presence of ants (*solid column*) and with *Ac. pisum* in the absence of ants (*open column*). **b** The number of *C. septempunctata* larvae climbing onto the plants with *Ap. craccivora* in the presence of ants (*solid column*) and with *Ac. pisum* in the absence of ants (*open column*)

lid under the plant with *Ac. pisum* in the absence of ants than on the petri dish lid under the plant with *Ap. craccivora* in the presence of ants (*Ap. craccivora*: $n = 30$, mean ± SE: 202.91 ± 28.22 s; *Ac. pisum*: $n = 30$, 493.31 ± 78.19, Mann–Whitney U-test, $z = -2.97$, $P = 0.0030$).

The number of ladybird larvae climbing onto the plants with *Ac. pisum* in the absence of ants was higher than that of the ladybird larvae climbing onto the plants with *Ap. craccivora* in the presence of ants (binomial test, $P = 0.0026$, Fig. 3b).

Choice of plants with *Ap. craccivora* or *Ac. pisum* after experiencing attacks by ants on the plant with *Ap. craccivora*

Although the number of ladybird larvae arriving at petri dish lids under the plants did not differ among plants ($\chi^2 = 0.52$, $P = 0.82$; Fig. 4a), significantly fewer larvae climbed onto plant C with *Ac. pisum* than onto plant A with *Ap. craccivora* ($\chi^2 = 6.53$, $P = 0.0161$; Fig. 4b). The number of ladybird larvae climbing onto plant A and plant B with *Ap. craccivora* did not differ ($\chi^2 = 0.10$, $P = 0.88$).

Discussion

Although the prey preference and searching behaviors of aphidophagous ladybird larvae have been investigated by many researchers, as mentioned in the Introduction, this study is the first to focus on the prey preference and foraging behaviors of the ladybird larvae as they are related to ant-attendance of the aphids.

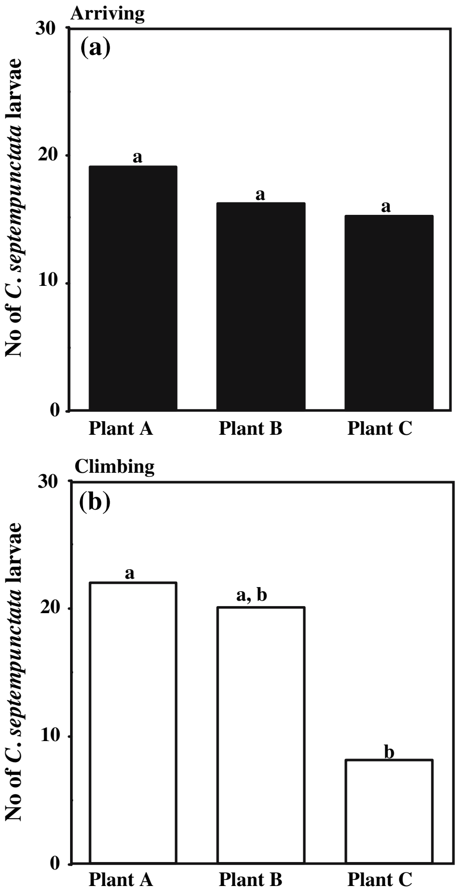

Fig. 4 The number of *C. septempunctata* larvae arriving at petri dish lids under the plants (**a**) and climbing onto the plant (**b**) in the experiment for the choice of plants after experiencing attacks by ants. *Plant A* The plant with *Ap. craccivora* at position A in the experimental apparatus, *plant B* the plant with *Ap. craccivora* at position B in the experimental apparatus, *plant C* the plant with *Ac. pisum* at position C in the experimental apparatus. *Different letters* indicate a significant difference (χ^2-test with Bonferroni adjustment for multiple tests, $P < 0.0167$)

Although Kalushkov and Hodek (2004) reported that *Ac. pisum* was a more suitable prey for *C. septempunctata* larvae than *Ap. craccivora* in the European populations, we were unable to determine any developmental and growth differences between *C. septempunctata* larvae that preyed on *Ac. pisum* and those that preyed on *Ap. craccivora*, indicating that the nutritional values of both aphid species for *C. septempunctata* larvae are similar in the population of western Japan.

This study showed that in the absence of ants, the foraging efficiency of the ladybird larvae that preyed on ant-tended *Ap. craccivora* was higher than that of the ladybird larvae that preyed on non-ant-tended *Ac. pisum* (Table 1). This underlying factor explaining this result was the fact that the number of non-ant-tended *Ac. pisum* that escaped by dropping off the plant per hour was conspicuously larger than that of ant-tended *Ap. craccivora* (Table 1). Murdoch and Marks (1973) reported a similar tendency in that the foraging efficiency of *C. septempunctata* larvae on a colony of ant-tended *Aphis* *fabae* was higher than that on a colony of non-ant-tended *Ac. pisum*. However, these authors did not discuss the difference in the predator avoidance manners between the two aphid species with reference to ant-attendance on the aphids.

The lack of difference between the number of *Ap. craccivora* that escaped by dropping off the plant in the presence of ants and those that escaped in the absence of ants (Table 1) may suggest that *Ap. craccivora* has a less well-developed escaping behavior pattern by dropping off plants because it is protected by ants from its natural enemies. Despite the loose relationship between *Ap. craccivora* and ants (facultative mutualism between aphids and ants), *Ap. craccivora* showed fewer escape behaviors, even in the absence of ants (Table 1). This may also be due to the fact that an increasing colony size without dispersion is likely to be advantageous for ant attraction (Bronstein 1994; Katayama and Suzuki 2002). In this study, the colony of *Ap. craccivora* was larger than that of *Ac. pisum* in the field.

The presence of ants on the plant with *Ap. craccivora* resulted in a shorter resident time of the ladybird larvae on the plant and a smaller number of aphids attacked and preyed upon (Table 1). Furthermore, in the experiment for the choice of plants with *Ap. craccivora* in the presence of ants or with *Ac. pisum* in the absence of ants (Experiment 3), the ladybird larvae were able to access the plant with *Ap. craccivora* less often because the larvae were frequently excluded by ants before climbing onto the plant. Thus, fewer ladybird larvae climbed onto the plant with *Ap. craccivora* in the presence of ants than onto the plant with *Ac. pisum* in the absence of ants (Fig. 3). This demonstrates a highly effective exclusion mechanism of the ladybird larvae by ants, as seen in Katayama and Suzuki (2002, 2003).

In the experiment for the choice of plants with *Ap. craccivora* or *Ac. pisum* by the ladybird larvae after experiencing attacks by ants on the plant with *Ap. craccivora* (Experiment 4), the ladybird larvae more frequently chose the plant with *Ap. craccivora* than that with *Ac. pisum* (Fig. 4b). This is due to the fact that *C. sepempunctata* larvae were able to choose the plant they wanted to climb by licking the honeydew that had fallen under the plant with aphids; consequently, they more frequently climbed onto the plant with *Ap. craccivora* than that with *Ac. pisum* (Ide et al. 2007). This result indicates that the ladybird larvae do not switch from foraging for *Ap. craccivora* to foraging for *Ac. pisum*, even after suffering attacks by ants on the plant with *Ap. craccivora* and thus being less able to prey on *Ap. craccivora*. This observation is supported by reports that ladybird larvae such as *C. septempunctata* larvae (Murdoch and Marks 1973) and *Adalia bipunctata* larvae (Hajek and Dahlsten 1987) foraging for a mixture of plural aphid species do not switch prey items.

The number of ladybird larvae climbing onto the plant with *Ap. craccivora* did not differ between plant A, on which the larvae had been previously attacked by ants, and plant B, on which the larvae had not been previously attacked by ants (Fig. 4). This result shows that the ladybird larvae are unable to remember where they have been previously attacked by ants. This lack of ability to learn and remember where they have been previously attacked may be due to the fact that ants do not always tend *Ap. craccivora* (facultative mutualism between aphids and ants), and thus the ladybird larvae have not been selected to avoid foraging for ant-tended aphids.

We conclude, therefore, that *C. septempunctata* larvae prefer foraging for colonies of *Ap. craccivora* to foraging for colonies of *Ac. pisum* because of a higher foraging efficiency for *Ap. craccivora* than *Ac. pisum*. This higher foraging efficiency is derived from the difference in the manner by which the aphids avoid attacks by natural enemies in association with ant-attendance by aphids. These results may explain why ladybird larvae in the field visited *V. angustifolia* plants with *Ap. craccivora* more frequently than *V. angustifolia* plants with *Ac. pisum* and why they made more visits when ants were absent than when they were present (Fig. 2).

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