The efficiency of herbivore exclusion by ants on the vetch *Vicia angustifolia* L. (Leguminosae), mediated by ant attraction to aphids

NOBUHIKO SUZUKI[1], KAZUHIRO OGURA AND NOBORU KATAYAMA[1]

Department of Biology, Faculty of Science, Kobe University, Tsurukabuto, 1-2-1, Nada, Kobe, 677-8501, Japan

[1] Present address: Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Honjo, 1, Saga, 840-8502, Japan

Address for correspondence: Nobuhiko Suzuki, Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Honjo, 1, Saga, 840-8502, Japan

e-mail: suzukin@cc.saga-u.ac.jp

fax: 0952-28-8792

telephone: 0952-28-8792

running title: herbivore exclusion by ants on the vetch

Abstract

The efficiency of herbivore exclusion by ants on the vetch *Vicia angustifolia* L. with

extrafloral nectary (EFN), mediated by ant attraction to aphids was investigated in the

field census and laboratory experiments. In the field, workers of *Lasius japonicus*

Santschi and *Tetramorium tsushimae* Emery frequently visited plants of the vetch

parasitized by aphids of *Aphis craccivora* Koch, but a few workers on plants

unparasitized by aphids. An increase in the number of ants visiting a plant with

increasing number of aphids caused in a decrease in the number of larvae of the weevil,

Hypera postica Gyllenhal. Therefore, the efficiency of herbivore exclusion by ants was

higher on plants parasitized by *Ap. craccivora* aphids than that on plants unparasitized by

aphids. In laboratory experiments, *L. japonicus* workers patrolled frequently not only

shoots with *Ap. craccivora* aphids but also shoots without them. On the other hand, *T.*

tsushimae workers visited mainly shoots with *Ap. craccivora* aphids but less frequently

on shoots without aphids. Therefore, *L. japonicus* workers excluded herbivores more

efficiently on plants of the vetch than *T. tsushimae* workers. Consequently, the

efficiency of herbivore exclusion by ants on the vetch may be influenced directly by

difference in ant species and indirectly by the presence of aphids on plants. This study

highlights the significance of indirect interactions between ants and plants with EFN,

mediated by ant attraction to aphids for herbivore exclusion of plants.

key words: *Aphis craccivora*; extrafloral nectary; honeydew; *Lasius japonicus*;

Tetramorium tsushimae

INTRODUCTION

It has been known that many plant species with extrafloral nectary (EFN) depend on ants

for herbivore exclusion (Bentley 1977b; Koptur 1992). Ants feed on extrafloral nectar,

including mainly sugars, and exclude herbivores from plants (Tilman 1978; Barton 1986;

Koptur & Lawton 1988; Apple & Feener 2001), resulting in a decrease in injury, and an

increase in the growth rate, survival rate, and reproductive success of the plants (Bentley

1977a; Inouye & Taylor 1979). However, some studies have insisted that benefits from

herbivore exclusion by EFN are not clear (O'Dowd & Catchpole 1983; Rashbrook *et al*.

1992). Thus, the adaptive significance of EFN has been controversial for decades

(Bentley 1977b; Beattie 1985; Koptur 1992).

Mutualistic interactions between ants and homopteran insects such as Aphididae,

Membracidae, Coccidae, and Pseudococcidae are well known (Way 1963; Carroll &

Janzen 1973; Katayama & Suzuki 2002). Homopterans produce honeydew for ants, and

ants exclude natural enemies of homopteran, including predators (Way 1963; Bristow 1984; Katayama & Suzuki 2002) and parasitoids (Buckley & Gullan 1991; Itioka & Inoue 1996).

In temperate regions, most of the mutualistic interactions between ants and plants with EFN and between ants and aphids are not one-to-one obligate relationships, but facultative relationships in which one aphid species or one plant species with EFN are attended by many ant species (Bristow 1984; Barton 1986; Cushman & Addicott 1989; Horvitz & Schemske 1990; Oliveira et al. 1999; Shingleton & Foster 2000), or one ant species utilizes the honeydew of many aphid species or the EFN of many plant species (Cushman & Addicott 1989; Sakata 1994, 1995, 1999; Völkl et al. 1999; Apple & Feener 2001; Fischer et al. 2001). Therefore, the intensity of the mutualistic interactions between ants and plants and between ants and aphids depends on various factors, such as multi-species interactions that consist of different species of plants, ants, and aphids, and the utilization of multi-resources by the ants.

Indirect interactions among plants without EFN, aphids and ants have been investigated by several authors (Messina 1981; Skinner & Whittaker 1981; Ito & Higashi 1991; Floate & Whitham 1994), and they showed that an increase in the number of ants visiting a plant with increasing number of aphids resulted in high efficiency of herbivore exclusion.

When examining ant-tended aphids that parasitize on plants with EFN, it is necessary to consider that not only direct interactions between ants and plants and between ants and aphids, but also indirect interactions among the plants, aphids and ants

occur. However, a few studies have elucidated these interactions (Sakata & Hashimoto 2000; Katayama & Suzuki 2003a).

In this study, the interactions of the vetch, *Vicia angustifolia* L. with EFN, the ants, the aphids that parasitize the vetch, and the herbivores that feed on the vetch were investigated in field and laboratory experiments. Katayama and Suzuki (2003a) showed that when aphids parasitized on plants with EFN, more ants were attracted to the honeydew excreted by the aphids than to the EFN secreted by plants. Thus, we would expect the efficiency of herbivore exclusion by ants to be higher on plants with ant-tended aphids than on plants without aphids. In order to test this hypothesis, we focused on how the efficiency of herbivore exclusion by ants was influenced by the ant species present on the plant, and by the presence of ant-tended aphids on plants with EFN. We discussed the significance of indirect interactions among plants, aphids and ants for herbivore exclusion from plants.

MATERIALS AND METHODS

The plant

The vetch, *V. angustifolia*, is an annual legume that grows mainly in open lands such as roadsides, grasslands, footpaths and wasted areas. In western Japan, the overwintering plants rapidly grow in early spring, and bear many EFN on the stipules. The plants anther in March to June, and produce pods which include five to eleven seeds successively from May to June.

Field census

A field census examining the abundance of herbivorous insects and aphids and ant activity on the vetch was carried out at two sites (site A: 640 m altitude; site B: 400 m altitude) mid-slope on Mt. Rokko, Kobe City, western Japan (34°41'N, 135° 11'E). Both sites consist of sunny, open land where many vetch plants are distributed in patches. At each site, 20 plants were randomly selected in early April, 2000, and marked individually using numbered flags.

At each site, the number of aphids and herbivorous insects on each plant, and the number of ants visiting each plant, were investigated every three days from late April to early June, when the plants bore flowers and produced pods successively. On each census day, the ants were counted five times at 10 min intervals on each plant, and the average obtained from these five counts was used for analysis.

Matured pods on each plant were collected in early June, and the number of seeds was counted in a laboratory.

Laboratory experiments

Two ant species, *Lasius japonicus* Santschi and *Tetramorium tsushimae* Emery, and one aphid species, *Aphis craccivora* Koch, all of which were frequently observed in the field census, were used in laboratory experiments.

The workers of *L. japonicus* are medium sized ants (about 4 mm in body length). They prefer sugars, and frequently feed on honeydew excreted by aphids (Völkl *et al.* 1999; Sakata & Hashimoto 2000) and on extrafloral nectar secreted by plants (Sakata &

Hashimoto 2000).   The *L. japonicus* workers are aggressive when other insects invade

their colony and feeding sites (Itioka & Inoue 1999).

The workers of *T. tsushimae* are small omnivorous ants about 2 mm in body

length.   They mainly forage insect carcasses, but also prefer sugars, such as the

honeydew of homopteran insects and the extrafloral nectar of plants.   When a worker of

T. tsushimae finds a preferable food resource, she recruits many colony members to that

resource.

Colonies of *L. japonicus* and *T. tsushimae* were collected at Kobe City.   Each of

these ant species was colonized in 10 test tubes (1.2 cm in diameter and 12 cm length),

each of which contained 200 individuals.   The bottom of each tube was packed with wet

cotton wool about 3 cm deep to maintain a suitable humidity level.   The tube was

covered with aluminum foil to maintain darkness as an ant nest.   To form an entrance,

each tube was connected to a vinyl chloride tube 6 mm in inner diameter and 10 cm long.

The ants were fed 10% sucrose solution, delivered via a test tube (1.2 cm in diameter, 12

cm long) plugged with cotton wool and reared in $25°C$ under a photoperiod of 24L0D.

The ants were starved for three days prior to the start of the experiments, in order

to increase the sensitivity of their reactions to the honeydew and extrafloral nectar.

The aphids of *Ap. craccivora* were collected in Kobe City and reared on the

seedlings of *V. faba* grown in polyethylene pots (9 cm in diameter, 8 cm deep) at 25°C

under a photoperiod of 24L0D.

Seeds of *V. angustifolia* stocked in a laboratory were soaked in H_2SO_4 for 30 min,

and after washing, the seeds were sown onto wet cotton in petri dishes at $10°C$ under a

photoperiod of 8L16D. The seedlings germinated from these seeds were transplanted individually into polyethylene pots 8 cm in diameter and 7.5 cm in depth, and cultivated at 10℃ under a photoperiod of 8L16D for three months. The seedlings were then transported at 20℃ under a photoperiod of 14L10D. The cultivated plants that grew to about 40 cm in height with six shoots were transplanted into a plastic pot with 10 cm in diameter and 4.5 cm in depth, which were filled with water. Each plastic pot was covered with a petri dish lid with a 15 mm hole in the center, to allow penetration by the plant's shoots. The plant was kept under a fluorescent lamp (100W) (®Toshiba, EFD23EN, Tokyo, Japan) hanging about 20 cm above the plant.

Aphids of *Ap. craccivora* were placed on top of three shoots of each plant using a paintbrush. A day later, we established the aphid density at 100 individuals per plant by removing the extra aphids from the plant. These aphids did not disperse across the shoots, and therefore three of six shoots on each plant were parasitized by aphids, but the other three shoots were not.

The first instar larvae of the silkworm, *Bombyx mori* L., were used as a model organism for the herbivores, because it was impossible to find enough herbivores with a similar body size in the field census to use in the experiments.

The silkworm eggs stocked at 5℃ were soaked in HCl at 48℃ for seven min and then washed and placed on wet cotton wool in petri dishes at 25℃ under a photoperiod of 24L0D. The hatchlings were then used for the experiments.

The entrances to each of the *L. japonicus* or *T. tsushimae* ant nests were placed on each pot, giving the ants a chance to freely visit the plant. The ants' activity stabilized 1

hr after their nest was placed on the pot. Once the ants' activity had stabilized, three 1st instar silkworm larvae were released on the shoots with or without aphids. Each experiment was repeated 10 times.

We recorded the proportion of silkworm larvae dropped by the ants from each plant during a 120 min time period, and the residence time of silkworm larvae on the plant. If silkworm larvae remained on the plant longer than 120 min, we completed the observation. The number of ants visiting shoots with and without aphids, and the number of ants utilizing the honeydew of the aphids and EFN of the plant, were counted at 5 min intervals during a 120 min period.

For statistical tests of variance, the proportions of silkworm larvae dropped by the ants were transformed as the arcsine of the square root.

RESULTS

Abundance of insects on the vetch in the field

At site A, *L. japonicus* workers were the most abundant ant species found, and the average number of workers per plant was 1.88 ± 0.18 (mean $\pm$ SE, n =280). Excepting the case that ants did not visit a plant, the average number of workers per plant was 3.84 ± 0.27 (mean $\pm$ SE, n = 137). Workers of *Formica japonica* Motschulsky were also sometimes found on the vetch. Three aphid species, *Ap. craccivora*, *Acyrthosiphon pisum* Harris and *Megoura crassicauda* Mordvilko, parasitized on the vetch, and their densities were 5.23 ± 0.86, 3.23 ± 0.42 and 2.54 ± 0.38 per plant (mean $\pm$ SE, n = 280), respectively.

At site B, the main ant species visiting the vetch was *T. tsushimae*, and the average number of workers per plant was 3.17 ± 0.29 (mean ± SE, n = 240). Excepting the case that ants did not visit a plant, the average number of workers per plant was 6.92 ± 0.42 (mean ± SE, n = 110). *Camponotus japonicus* Mayr and *F. japonica* workers were occasionally found on the vetch. Three aphid species, *Ap. craccivora*, *Ac. pisum* and *M. crassicauda*, were parasitized on the vetch, and their densities were 1.83 ± 0.38, 1.12 ± 0.29 and 5.75 ± 0.73 per plant (mean ± SE, n = 240), respectively.

Larvae of the weevil, *Hypera postica* Gyllenhal, which feed on flower buds, flowers and leaves of the vetch were found at both sites A and B. The average number of weevil larvae per plant at sites A and B was 1.13 ± 0.09 (n = 280) and 1.10 ± 0.10 (n = 240) (mean ± SE), respectively. Lepidopteran larvae were occasionally found on the vetch at both sites A and B. Predators to aphids, syrphid larvae (sites A and B) and the larvae of the ladybeetle, *Coccinella septempunctata* L., (site B) were sometimes found on the vetch.

Correlations among abundance of aphids, ants and herbivores and seed production in the field

At site A, the number of *L. japonicus* workers found on a plant was positively correlated to the number of *Ap. craccivora* aphids found on that plant (Fig.1a, $r = 0.743$, $P < 0.0001$). However, hardly any *L. japonicus* workers were found on plants parasitized by *Ac. pisum* and *M. crassicauda* aphids. At site B, the number of *T. tsushimae* workers found on a plant was positively correlated to the number of *Ap. craccivora* aphids found on that plant

Fig. 1

(Fig.1b, $r = 0.442$, $P < 0.0001$), but not to the number of *Ac. pisum* ($r = 0.019$, $P = 0.77$) and *M. crassicauda* ($r = 0.122$, $P = 0.06$) aphids found on it.

The cumulative number of the weevil larvae on each plant was negatively correlated to the cumulative number of *L. japonicus* workers on each plant throughout the entire census at site A (Fig. 2, $r = 0.468$, $P = 0.0432$), but not to that of *T. tsushimae* at site B ($r = 0.155$, $P = 0.51$).

Fig. 2

The number of seeds per plant was negatively correlated to the cumulative number of the weevil larvae on each plant throughout the entire census (Fig. 3, $r = 0.483$, $P = 0.0016$), but not to the cumulative number of aphids of three species on each plant ($r = 0.012$, $P = 0.94$).

Fig. 3

Number of ants visiting shoots and utilizing EFN and honeydew in relation to the presence of aphids on shoots in laboratory experiments

The average numbers of *L. japonicus* and *T. tsushimae* workers per plant were 18.10 ± 1.16 (mean $\pm$ SE, n = 10) and 14.41 ± 1.25 (mean $\pm$ SE, n = 10), respectively. The visiting and foraging activity of *L. japonicus* and *T. tsushimae* workers on shoots with and without *Ap. craccivora* aphids are shown in Table 1. The cumulative number of workers visiting shoots throughout 24 counts during the 120 min observation period significantly differed between ant species and between shoots with and without aphids (two-way ANOVA; ant: $F = 5.004$, $P = 0.0316$, aphid: $F = 155.035$, $P < 0.0001$, ant x aphid: $F = 5.233$, $P = 0.0281$, Table 1). The cumulative number of workers of both species on shoots with aphids was significantly larger than that on shoots

Table 1

without aphids (*t*-test, adjusted by Bonferroni procedure as multiple comparison, *P* <

0.001), and on shoots without aphids, that of *T. tsushimae* was significantly smaller than

that of *L. japonicus* (*t*-test, adjusted by Bonferroni procedure as multiple comparison, *P* <

0.001).

The cumulative number of workers utilizing EFN throughout 24 counts during the

120 min observation period significantly differed between shoots with and without aphids,

but there was no difference between ant species (two-way ANOVA; ant: *F* = 3.689, *P* =

0.0627, aphid: *F* = 20.254, *P* < 0.0001, ant x aphid: *F* = 2.308, *P* = 0.1374, Table 1).

The cumulative number of *L. japonicus* workers utilizing EFN on shoots with aphids was

significantly smaller than on shoots without aphids (*t*-test, adjusted by Bonferroni

procedure as multiple comparison, *P* < 0.01).

The cumulative number of workers utilizing honeydew throughout 24 counts

during the 120 min observation period did not significantly differ between ant species

(Mann-Whitney *U*-test; *z* = -0.227, *P* = 0.8205, Table 1).

The efficiency of herbivore exclusion by ants from shoots in laboratory experiments

The exclusion efficiency of silkworm larvae from shoots by workers of both species is

shown in Table 2. The proportion of silkworm larvae dropped by ants during the 120

min observation period significantly differed between ant species and between shoots
Table 2

with and without aphids (two-way ANOVA; ant: *F* = 37.949, *P* < 0.0001, aphid: *F* =

79.261, *P* < 0.0001, ant x aphid: *F* = 29.972, *P* < 0.0001). The proportion of silkworm

larvae dropped by *T. tsushimae* workers on shoots without aphids was significantly lower

than both that by *T. tsushimae* workers on shoots with aphids (*t*-test, adjusted by Bonferroni procedure as multiple comparison, $P < 0.001$) and that by *L. japonicus* workers on shoots without aphids (*t*-test, adjusted by Bonferroni procedure as multiple comparison, $P < 0.001$).

The residence time of silkworm larvae that remained on the shoots until dropped by the ants significantly differed between ant species and between shoots with and without aphids (two-way ANOVA; ant: $F = 6.646$, $P = 0.0118$, aphid: $F = 13.872$, $P = 0.0004$, ant x aphid: $F = 4.295$, $P = 0.0414$). The residence time of silkworm larvae that remained on the shoots until dropped by *T. tsushimae* workers on shoots without aphids was significantly longer than both that by *T. tsushimae* workers on shoots with aphids (*t*-test, adjusted by Bonferroni procedure as multiple comparison, $P < 0.01$) and that by *L. japonicus* workers on shoots without aphids (*t*-test, adjusted by Bonferroni procedure as multiple comparison, $P < 0.05$).

DISCUSSION

Relationships between aphids and ants

In the field census, the number of *L. japonicus* and *T. tsushimae* workers visiting a plant was positively correlated to the number of *Ap. craccivora* aphids parasitized on the plant (Fig. 1). In many ant species, it has been known that increased density of aphids on a plant results in an increase in the number of ants recruited to that plant (for example, Floate & Whitham 1994, Sakata 1995). However, *L. japonicus* workers seldom visited plants parasitized by *Ac. pisum* and *M. crassicauda* aphids, and the number of *T.*

tsushimae workers on a plant was not correlated to the number of *Ac. pisum* and *M. crassicauda* aphids on a plant. These results indicate that *Ap. craccivora* is an ant-tended aphid, and that *Ac. pisum* and *M. crassicauda* are not. Therefore, *L. japonicus* and *T. tsushimae* workers are likely to more frequently visit plants parasitized by *Ap. craccivora* aphids than plants parasitized by *Ac. pisum* and *M. crassicauda* aphids, and plants unparasitized by aphids.

Effects of herbivory on seed production

The negative correlation between the number of seeds per plant and the cumulative number of the weevil larvae (Fig. 3), and no correlation between the number of seeds per plant and the cumulative number of aphids, are likely to imply that injury by the weevil larvae rather than aphids brings about damage to the seed production capabilities of the vetch. This may be caused by direct injury to reproductive organs such as the flower buds and flowers by the feeding of the weevil larvae, compared to indirect injury by the sucking on non-reproductive organs, such as stems and leaves, by the aphids.

An increase in the number of ants visiting a plant with increasing number of aphids caused in a decrease in the number of weevil larvae (Figs. 1 and 2). Therefore, sucking by several dozens of aphids per plant, as found in this study, is likely to cause minor damage, and the presence of aphids on a plant may be beneficial for the plant, by increasing the number of ants attracted to them. On plants without EFN, similar results have been reported by several authors (Messina 1981; Skinner & Whittaker 1981; Ito & Higashi 1991; Floate & Whitham 1994).

Utilization of EFN and honeydew by ants

The frequency of EFN utilization by workers of both species was conspicuously lower on shoots with *Ap. craccivora* aphids than without (Tables 1). However, workers of both species frequently utilized the honeydew of aphids. Similar results were reported in *V. faba* with and without aphids of *Ap. craccivora* (Katayama & Suzuki, 2003a). This may be caused by ant preferring the more abundant amounts of carbon or nitrogen found in honeydew than EFN, as was expected by Katayama and Suzuki (2003a) based on data obtained on the concentration of sugars or amino acids in EFN or honeydew (e.g. Koptur 1979, Fischer *et al*. 2002).

Therefore, the presence of ant-tended aphids on plants is likely to influence the frequency of ant visits, even on plants that would be able to attract ants by their EFN.

Foraging activities of ants on shoots

The number of workers of both species per plant in the field census was less than in the laboratory experiments, because the workers extensively foraged on many plants of the vetch in the field. Therefore, the foraging activity in the laboratory experiments may be overestimated, compared with that in the field.

The number of workers of *L. japonicus* and *T. tsushimae* on shoots with aphids was larger than that on shoots without aphids (Table 1), because the ants were attracted to the honeydew produced by the aphids. Although the number of workers of *L. japonicus* was nearly equal to that of *T. tsushimae* on shoots with aphids, there were more *L.*

japonicus workers than *T. tsushimae* workers on shoots without aphids. This shows the difference in foraging behavior between *L. japonicus* and *T. tsushimae*. Workers of *L. japonicus* foraged actively on all shoots, including shoots without aphids, but workers of *T. tsushimae* intensively foraged on shoots with aphids, but less frequently on shoots without aphids. These results would be supported by Katayama & Suzuki (2003b), in which the number of workers visiting a plant of *V. fava* with EFN did not differ between *L. japonicus* and *T. tsushimae*, but *L. japonicus* workers foraged on a plant more extensively than *T. tsushimae*. Extrafloral nectar may be a more preferable resource for *L. japonicus* than *T. tsushimae* which is an omnivorous ant. However, this expectation should be tested in future studies by comparing the foraging behaviors of both ant species on plants without EFN.

Efficiency of herbivore exclusion by ants

The difference in foraging behaviors between *L. japonicus* and *T. tsushimae* may result in difference in the efficiency of herbivore exclusion. However, a few studies have elucidated the difference in the efficiency of herbivore exclusion by different ant species in relation to their foraging behaviors (Gaume & McKey 1999).

In this study, *L. japonicus* workers excluded herbivores more actively than *T. tsushimae* workers (Table 1). This is mainly due to the fact that *L. japonicus* exhibits higher foraging activity than *T. tsushimae* on shoots without aphids. On shoots with aphids the number of workers and the efficiency of herbivore exclusion in *L. japonicus* were nearly equal to those in *T. tsushimae*. On shoots without aphids, however, there

were more *L. japonicus* workers than *T. tsushimae* workers, and the efficiency of herbivore exclusion by *L. japonicus* was higher than that by *T. tsushimae*. This may be caused by the higher probability of encounter between herbivores and *L. japonicus* workers than *T. caespitum* workers. The fact that *L. japonicus* workers dropped silkworm larvae more efficiently than *T. tsushimae* workers on shoots without aphids is not likely to link with ant aggressiveness against herbivores, because on shoots with aphids, the number of ants and the proportion of the silkworm larvae dropped from a plant were nearly equal between *L. japonicus* and *T. tsushimae* (Table 1). Katayama and Suzuki (2003b) also reported no difference in aggressiveness between *L. japonicus* and *T. tsushimae*.

In the field census, the average numbers of weevil larvae per plant did not differ between site A (presence of *L. japonicus*) and site B (presence of *T. tsushimae*). This may be caused by the fact that the number of *T. tsushimae* workers visiting a plant was much more than that of *L. japonicus*.

In conclusion, the efficiency of herbivore exclusion by ants on plants may be influenced directly by the difference in ant species and indirectly by the presence of aphids, and the differences in aphid species. This study highlights the significance of indirect interactions between ants and plants with EFN, mediated by ant attraction to aphids for the herbivore exclusion of plants. Furthermore, the balance in costs of sucking by aphids, and the benefits of ant attraction for plants, may be important for herbivore exclusion by ants.

ACKNOWLEDGEMENTS

REFERENCES

APPLE J. L. & FEENER JR. D. L. (2001) Ant visitation of extrafloral nectaries of

Passiflora: the effects of nectary attributes and ant behavior on patterns in

facultative ant-plant mutualisms. Oecologia 127: 409-416.

BARTON A.M. (1986) Spatial variation in the effect of ants on an extrafloral nectary plant.

Ecology 67: 495-504.

BEATTIE A. J. (1985) The evolutionary ecology of ant-plant mutualisms. Cambridge

University Press, New York.

BENTLEY B. L. (1977a) The protective function of ants visiting the extrafloral nectaries of

Bixa orellana (Bixaceae). Journal of Ecology 65: 27-38.

BENTLEY B. L. (1977b) Extrafloral nectaries and protection by pugnacious bodyguards.

Annual Review of Ecology and Systematics 8: 407-427.

BRISTOW C. M. (1984) Differential benefits from ant attendance to two species of

Homoptera on New York ironweed. Journal of Animal Ecology 53: 715-726.

BUCKLEY R. & GULLAN P. (1991) More aggressive ant species (Hymenoptera:

Formicidae) provide better protection for soft scales and mealybugs (Homoptera:

Coccidae, Pseudococcidae). Biotoropica 23: 282-286.

CARROLL C. R. & JANZEN D. H. (1973) Ecology of foraging ants. Annual Review of

Ecology and Systematics 4: 231-257.

CUSHMAN J. H. & ADDICOTT J. F. (1989) Intraspecific and interspecific competition for

mutualists: ants as a limited and limiting resource for aphids. Oecologia 79:

315-321.

FISCHER M. K., HOFFMANN K. H. & VÖLKL W. (2001) Competition for mutualists in

an ant-homopteran interaction mediated by hierarchies of ant attendance.

Oikos **92**: 531-541.

FISCHER M. K., VÖLKL W., SCHOPF R., HOFFMANN K. H. (2002) Age-specific

patterns

in honeydew production and honeydew composition in the aphid *Metopeurum*

fuscoviride: implications for ant-attendance. *Journal of Insect Physiology*

48: 319-326.

FLOATE K. D. & WHITHAM T. G. (1994) Aphid-ant interaction reduces chrysomelid

herbivory in a cottonwood hybrid zone. *Oecologia* **97**: 215-221.

GAUME L. & MCKEY D. (1999) An ant-plant mutualism and its host-specific parasite:

activity rhythms, young leaf patrolling, and effects on herbivores of two

specialist plant-ants inhabiting the same myrmecophyte. *Oikos* **84**: 130-144.

HORVITZ C. C. & SCHEMSKE D. W. (1990) Spatiotemporal variation in insect mutualists

of a neotropical herb. *Ecology* **71**: 1085-1097.

INOUYE D. W. & TAYLOR O. R. (1979) A temperate region plant-ant-seed predator

system: consequences of extrafloral nectar secretion by *Helianthella*

quinquenervis. *Ecology* **60**: 1-7.

ITO F. & HIGASHI S. (1991) An indirect mutualism between oaks and wood ants via

aphids. *Journal of Animal Ecology* **60**: 463-470.

ITIOKA T. & INOUE T. (1996) Density-dependent ant attendance and its effects on the

parasitism of a honeydew-producing scale insect, *Ceroplastes rubens*. *Oecologia* **106**: 448-454.

ITIOKA T. & INOUE T. (1999) The alternation of mutualistic ant species affects the population growth of their trophobiont mealybug. *Ecography* **22**: 169-177.

KATAYAMA N. & SUZUKI N. (2002) Cost and benefit of ant attendance for *Aphis craccivora* (Hemiptera: Aphididae) with reference to aphid colony size. *The Canadian Entomologist* **134**: 241-250.

KATAYAMA N. & SUZUKI N. (2003a) Changes in the utilization of extrafloral nectaries of *Vicia faba* (Leguminosae) and honeydew of aphids by ants with increasing aphid density. *Annals of the Entomological Society of America* **96**: 579-584.

KATAYAMA N. & SUZUKI N. (2003b) Bodyguard effects for the aphids of *Aphis craccivora* Koch (Homoptera: Aphididae) as related to the activity of two ant species, *Tetramorium caespitum* and *Lasius niger* L. (Hymenoptera: Formicidae). *Applied Entomology and Zoology* **38**: 427-433.

KOPTUR S. (1979) Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *American Journal of Botany* **66**: 1016-1020.

KOPTUR S. (1992) Extrafloral nectary-mediated interactions between insects and plants. In: *Insect-plant interaction Vol. IV.* (ed E. BERNAYS) pp.81-129. CRC Press, Boca Raton.

KOPTUR S. & LAWTON J. H. (1988) Interactions among vetches bearing extrafloral nectaries, their biotic protective agents, and herbivore. *Ecology* **69**: 278-283.

MESSINA F. J. (1981) Plant protection as a consequence of an ant-membracid

mutualism: interactions on goldenrod (*Solidago* sp.). *Ecology* **62**: 1433-1440.

O'DOWD D. J. & CATCHPOLE E. A. (1983) Ants and extrafloral nectaries: no evidence for

plant protection in *Helicrysum* spp. – ant interactions. *Oecologia* **59**: 191-200.

OLIVEIRA P. S., RICO-GRAYM V., DÍAZ-CASTELAZO C., CASTILLO-GUEVARA C. (1999)

Interaction between ants, extrafloral nectaries and insect herbivores in

Neotropical coastal sand dunes; herbivore deterrence by visiting ants increases

fruit set in *Opuntia stricta* (Cactaceae). *Functional Ecology* **13**:623-631.

RASHBROOK V. K., COMPTON S. G., LAWTON J. H. (1992) Ant-herbivore interactions:

reasons for the absence of benefits to a fern with foliar nectaries. *Ecology* **73**:

2167-2174.

SAKATA H. (1994) How an ant decides to prey on or to attend aphids. *Researches on

Population Ecology* **36**: 45-51.

SAKATA H. (1995) Density-dependent predation of the ant *Lasius niger* (Hymenoptera:

Formicidae) on two attended aphids *Lachnus tropicalis* and *Myzocallis kuricola*

(Homoptera: Aphididae). *Researches on Population Ecology* **37**: 159-164.

SAKATA H. (1999) Indirect interactions between two aphid species in relation to ant

attendance. *Ecological Research* **14**: 329-340.

SAKATA H. & HASHIMOTO Y. (2000) Should aphids attract or repel ants? Effect of rival

aphids and extrafloral nectaries on ant-aphid interactions. *Population Ecology*

42: 171-178.

SHINGLETON A. W. & FOSTER W. A. (2000) Ant tending influences soldier production in

a social aphid. *Proceedings of the Royal Society of London B* **267**: 1863-1868.

SKINNER G. J. & WHITTAKER J. B. (1981) An experimental investigation of inter-relationships between the wood-ant (*Formica rufa*) and some tree-canopy herbivores. *Journal of Animal Ecology* **50**: 313-326.

TILMAN D. (1978) Cherries, ants, and tent caterpillars: Timing of nectar production in relation to susceptibility of caterpillars to ant predation. *Ecology* **59**: 686-692.

VÖLKL W., WOODRING J., FISCHER M., LORENZ M. W. & HOFFMANN K. H. (1999) Ant-aphid mutualism: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* **118**: 483-491.

WAY M. J. (1963) Mutualism between ants and honeydew-producing Homoptera. *Annual Review of Entomology* **8**: 307-344.

Figure legends

Fig. 1   The relationships of the number of ants of *L. japonicus* (a) and *T. tsushimae* (b) to

the number of aphids of *Ap. craccivora* on a plant of the vetch.

Fig.2   The relationship of the cumulative number of the weevil larvae to the

cumulative number of workers of *L. japonicus* on a plants of the vetch throughout the

entire census.

Fig. 3   The relationship of the number of seeds per plant to the cumulative number of

the weevil larvae on a plant of the vetch throughout the entire census.