

Costs and benefits of ant attendance to the drepanosiphid aphid *Tuberculatus quercicola*

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The defensive effects of ants against aphid predators have been well documented in the mutualistic relationship of aphids and their attending ants. However, it is not clear whether ant attendance has any direct effect on the aphids' growth and reproduction. Through field experiments, this study evaluates the benefits and, in particular, the costs of ant attendance to aphid colonies, focusing on the drepanosiphid aphid *Tuberculatus quercicola* which is associated with the Daimyo oak, *Quercus dentata*, and which is always attended by the red wood ant *Formica yessensis*. Ant attendance was clearly beneficial to the aphid; the exclusion of ants led to a significant increase in the extinction rate of aphid colonies. However, MANOVA and randomized block ANOVA indicated that in colonies continuously attended by ants, aphids had significantly smaller body size and produced a smaller number of embryos than in colonies isolated from ants when they were reared under homogeneous host conditions free from natural enemies. Thus, ant attendance had a negative influence on the growth and reproduction of the aphids, even though it contributed to the greater longevity of the aphid colonies. We hypothesize that ant-attended aphids are under intense selective pressures that act against aphid clones which fail to attract many ants, so that aphids have developed an adaptive mechanism to allocate a larger fraction of resources to the honeydew when they are requested to do so by the ants in order to ensure the ants' consistent visitation.

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For an understanding of the evolutionary dynamics of mutualism, it is indispensable to quantify the fitness costs and benefits resulting from the interaction with the partner species. The relationships between ants and homopterans or between ants and the larvae of lycaenid butterflies are among examples of mutualism that have been studied in great detail (Way 1963, Sudd 1987, Baylis and Pierce 1992, Dixon 1998 for reviews). These ant-attended insects obtain protection from the ants against natural enemies, pathogens, and unfavorable physical conditions by offering sugars and nitrogen-rich liquids to the ants as rewards. A number of studies have confirmed the effectiveness of ant attendance against natural enemies (Banks and Macaulay 1967,

Tilles and Wood 1982, Bristow 1984, Pierce et al. 1987, Devries 1991, Völkl 1992, Itioka and Inoue 1996, Stechmann et al. 1996).

The actual impact of ant attendance on the fitness of the partner species under conditions free from natural enemies remains controversial. Even among studies wherein identical or closely related species are used as the partner of an ant species, the results have often been inconsistent. In the aphid *Aphis fabae* Scopoli, while the reproductive and developmental rates have been reported to increase when the aphids are attended by ants (El-Ziady 1960), Stadler and Dixon (1998) revealed that ant attendance had significantly negative effects on the aphid's reproductive rate. Similarly, in

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some lycaenids ant attendance leads to weight loss in the larvae (Pierce et al. 1987) or a prolongation of developmental duration (Pierce et al. 1987, Robbins 1991), whereas in other lycaenids ant behaviors express no effect on larval performance, or even result in a significant gain in weight (Pierce and Easteal 1986, Fiedler and Hölldobler 1992, Wagner 1993, Cushman et al. 1994, Fiedler and Hummel 1995, Wagner and del Rio 1997). These results support the hypothesis that the outcomes of mutualistic interactions are basically variable and conditional (Cushman and Whitham 1989, Cushman 1991, Cushman and Addicott 1991).

This discrepancy in experimental results may depend on the studies' settings because ant attendance has multiple effects on the growth or reproduction of the partner species, making it difficult to control more than one factor at a time. It has been reported that ant attendance enhances aphids' feeding and excretion rates (Banks and Nixon 1958, Takeda et al. 1982), chemically delays the appearance of alate aphids (El-Ziady and Kennedy 1956), changes the distribution of aphids on the host plant (Banks 1958), and "tranquilizes" the activity of aphids and lycaenid larvae (El-Ziady 1960, Way 1963, Wagner and del Rio 1997). Thus, attending ants could affect the fitness of the partner not only through direct stimuli or manipulation but also indirectly by means of induced behavioral and physiological changes in the partner. To clarify the effects of tactile or chemical stimuli from attending ants in an experimental setting, it is of prime importance to maintain the uniformity of other ant-induced effects.

The present study attempts to quantify the attending ants' direct costs and benefits to the aphid *Tuberculatus quercicola* (Matsumura), a non host-alternating aphid associated with the Daimyo oak, *Quercus dentata* Thunberg, under the condition that natural enemies are present or absent, using an experimental design to cancel out the indirect effects of the attending ants.

An indirect effect of the experimental removal of attending ants seems to result mainly from (1) the increased movement of aphids within the host plant (Banks 1958), and (2) the increased proportion of nymphs developing into alates (El-Ziady and Kennedy 1956, Banks 1958). When within a host plant resources are heterogeneous, the dispersal of aphids from suitable feeding sites may result in decreased reproductive rates. Thus, in the present study, to reduce the heterogeneity of the host plants, aphid colonies were reared on single host leaves by netting the colonies. Furthermore, the heterogeneity between branches or trees was statistically adjusted using block designs. Because all nymphs of *T. quercicola* develop into alate viviparous females during spring and summer, it is not necessary to consider the effects of wing polymorphism. The outcomes of ant-attendance and ant-exclusion experiments will be discussed in terms of the exploitation of aphids by ants rather than in terms of mutualism.

Study area and materials

Observations and experiments were conducted on the Ishikari Coast, Hokkaido, northern Japan in 1998. Colonies of the red wood ant *Formica yessensis* Forel were found throughout the dunes, along which were spread bushy stand of the Daimyo oak, *Quercus dentata*. Nests of *F. yessensis* were distributed contagiously along this coast (Higashi and Yamauchi 1979).

On oaks that grow on dunes, *T. quercicola* colonies were always attended by the red wood ant *F. yessensis*, which never hunted the aphids during our observations.

Methods

Effects of ant attendance in the presence of predators

The effects of ants and predators on the growth of aphid populations were examined on four oak trees averaging 1.7 m tall. To remove the effects of genetic differences among the aphids on the results, prior to the experiment one aphid clone was reared on each of the study trees. On 28 May 1998, one fourth-instar nymph was collected on each study tree and transferred to a leaf on that tree using a small brush. The leaf was bagged with a nylon net (33 × 22 cm) to propagate the clone for about two months. In late July 1998 a total of 22 branches, each bearing a pair of shoots, were selected from the four trees. On each selected shoot, all leaves except one were cut and all aphids found on the remaining leaf were removed. Four clonal fourth-instar nymphs were transferred onto each branch's two remaining leaves on 28 July 1998. The leaf blades of *Q. dentata* are so large, ca 20 cm, as to accommodate a maximum of 200 aphids. After the transfer all the leaves were bagged with a nylon net in order to settle the aphids on the leaf and propagate the clone. After one week the bags were removed. For a pair of shoots on each branch, the following treatments were randomly allocated: (1) "ant-exclusion" treatment, in which the shoots were isolated by smearing ant repellent (Fuji-Tangle®) at the base to exclude ants but to allow the access of winged predators, and (2) "ant-attending" treatment, in which the shoots were kept intact to allow ant foraging and predation by insects and spiders. Several ants were always foraging on the study trees, so that on all "ant-attending" shoots ants found the aphids and started attending them a few minutes after the removal of bags. The number of surviving aphid colonies and the number of predators present were censused almost every day from 4 August to 2 September 1998 for a total of 26 censuses.

Failure-time analysis was applied to the survival rate of ant-excluded and ant-attended colonies to test the effects of attending ants on colony persistence. Further-

more, logistic regression was conducted to elucidate the difference in the proportion of colonies attacked between treatments, with census dates and treatments assigned to independent variables.

Effects of ant attendance in predator-free environments

The effects of ant attendance on aphid body size and reproduction were evaluated in a predator-free environment using three trees averaging 1.7 m tall. In this experiment, clonal aphids were used to compare the effects of different treatments. For this purpose, one aphid clone was propagated prior to the experiment on each study tree. On 28 May 1998, one fourth-instar nymph from a study tree was transferred to a leaf on that tree, and the leaf was bagged with a nylon net (33×22 cm) to propagate the clone for about two months. In late July 1998, from each of the trees seven branches, each with a pair of shoots, were randomly selected and used for the experiments. All the leaves but one were cut from each shoot, so that two leaves were left on each branch. Each pair of leaves was situated at the apex of the branch, forming a Y-shaped twig. All aphids were removed, and four clonal fourth-instar nymphs were transferred onto each leaf. Using the pair of leaves, ant-attended and ant-excluded colonies were established. After the transfer, one of the leaves was bagged with a nylon net to prevent predation (ant-excluded colonies). On the other leaf, two plastic tubes, each 6 cm long and 4 mm at the inside diameter, were attached with plastic tape along the petiole, and a net was bound over the tubes (ant-attended colonies). This treatment enabled ants alone to approach the aphid colony directly through the tubes.

Crowding is the primary factor affecting the morphological, physiological, and reproductive characteristics of aphids (Dixon 1969, Murdie 1969). Thus, to keep aphid population density constant on the areas of the study leaves, at each census some individuals were removed for adjustment of the number of aphids to a range of 30–50 individuals per leaf. Honeydew on the ant-excluded leaves was removed every three days.

To determine the exact number of embryos the aphids contained, only fourth-instar nymphs were collected from each bagged leaf from 23 July to 31 August 1998. All aphids were preserved in vials of 70% ethanol. Since the growth and reproduction of aphids depend strongly on the growth of their host plants (Kennedy et al. 1950, Mittler 1958, Dixon 1970), the time of collection is an important index of the host conditions. Thus, the aphids collected were categorized into two groups depending on collection dates, i.e. season 1 (from 23 July to 11 August) and season 2 (from 12 August to 31 August) of the same length (20 d). The following measurements were made for each aphid as indices of

growth and reproduction: body width, hind femur length, and numbers of mature and all embryos. The entire set of embryos included mature and immature embryos, and the two kinds of embryos were identified by the presence or absence of pigmented eyes. The dimensions of the body parts were measured using an eyepiece micrometer installed in a binocular microscope, and the numbers of mature and immature embryos contained in each female were counted after dissection. In calculating the statistical probability of the effect of a treatment, the use of several samples from one treatment unit often leads to pseudoreplication, which confounds the effect of the treatment with the local effect of the unit, resulting in an inflated d.f. number (Hurlbert 1984). To surmount this problem, the scores of all measurements of each colony were averaged for each season, and the averages were used for analyses. Branches on which either of the aphid colonies became extinct due to accidental invasions of predators or deterioration in host quality were not included in the analysis, for which consequently 19 branches and 12 branches were used for season 1 and season 2, respectively. In calculating the averages, 23.6 aphids per leaf (range 2–64, $SD \pm 15.8$) and 10.7 aphids per leaf (range 2–38, $SD \pm 9.5$) were used for season 1 and season 2, respectively.

Study trees and branches were assigned to blocks because aphid growth and reproduction are likely to be affected by the uncontrolled effects of individual variation of trees and branches as sources of systematic errors. Because the two leaves chosen in a block (branch) were close to each other and of the same size, effects peculiar to single leaves are considered to be minimized. In this randomized block design, the interaction terms including those relating to branches and trees were included in the error term (Sokal and Rohlf 1995). In this experimental design which includes four response variables, performing ANOVAs repeatedly for respective response variables inflates the probability of a type I error. To avoid this problem, we conducted the MANOVA-protected ANOVA procedure (Scheiner 1993). MANOVA was first performed to test the overall effect of attending ants on the relationships among the response variables. If and only if a significant difference was found between types of treatment in the MANOVA model, then randomized block ANOVA was subsequently conducted to test the effect of the treatment on each response variable. Furthermore, sequential Bonferroni tests (Rice 1989) were carried out to adjust *P* values in simultaneous comparisons of treatments or seasons throughout the randomized block ANOVAs. The MANOVA model contained “tree” and “branch nested within tree” as blocks, “treatment” and “season” as main effects and the interaction between season and treatment. The main effects were treated as fixed variables. Four statistics – Wilk’s lambda, Pillai’s trace, Hotelling-Lawley trace, and Roy’s greatest root

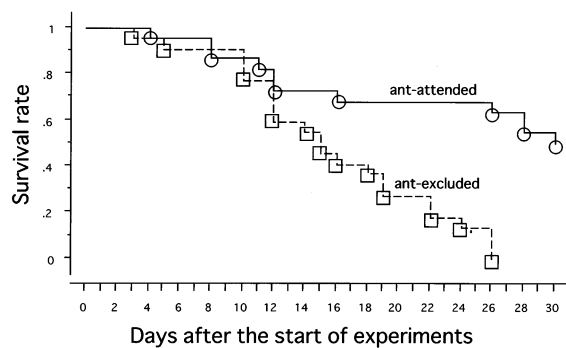


Fig. 1. Survival rates of ant-excluded and ant-attended colonies. In the ant-excluded treatment, colonies were isolated by smearing ant repellent 'tanglefoot' at the base to exclude ants but allow the access of winged predators. Ant-attended colonies were kept intact to allow ant foraging and predation by insects and spiders.

– are usually used in testing MANOVA models. Of the statistics, Pillai's trace statistic was chosen because of its robustness against violations of assumptions (Scheiner 1993). Because the interaction between season and treatment was not significant (MANOVA, numerator d.f. = 4, denominator d.f. = 36, $F = 0.805$, $P = 0.530$), MANOVA was conducted again after the interaction term was pooled with the error term (Sokal and Rohlf 1995). Because sample size was unequal among trees, among branches or between seasons, Type II sum of squares was consistently used in the SAS procedure GLM. Computations using the SAS program package (SAS 1990) were made in the Computing Center, Hokkaido University.

Table 1. Number of predators observed on leaves from which ants were excluded and on leaves where ants were allowed. The number of colonies in which predators were found in parentheses.

Predator	Ant-excluded (22 colonies)	Ant-attended (22 colonies)
Araneae		
<i>Clubiona</i> sp.	55 (15)	1 (1)
Acari		
<i>Erythraeus</i> sp.	0	1 (1)
Thysanoptera		
<i>Aeolothrips</i> sp.	2 (1)	0
Neuroptera		
<i>Mallada</i> sp. (larva)	5 (1)	0
Diptera		
<i>Episyrphus balteatus</i> (larva)	13 (6)	15 (8)
Dolichopodidae gen. sp.	1 (1)	0
Aculeata		
<i>Pemphredon</i> sp.	2 (2)	0
Coleoptera		
<i>Coccinella septempunctata</i>	2 (2)	2 (2)
Total no. of predators	80	19

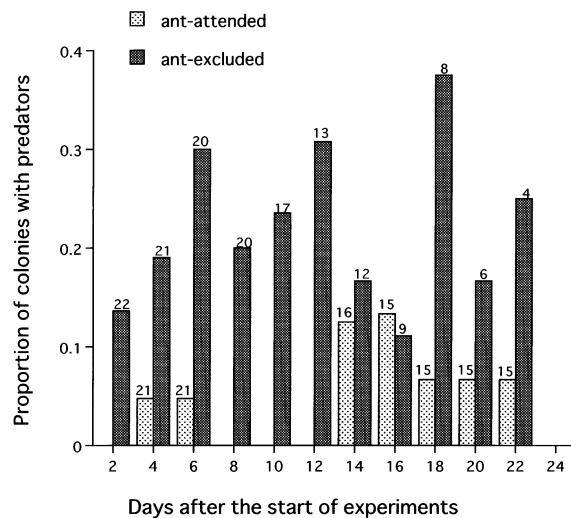


Fig. 2. Proportion of colonies invaded by predators in the ant-excluded treatment and ant-attended treatment. Numbers indicate the total number of colonies that had survived until that time.

Results

Effects of ant attendance on the presence of predators

Formica yessensis continued to visit all aphid colonies on the study trees throughout the study period. The experimental results demonstrated the significantly greater longevity of the aphid colonies under ant attendance. None (0%) of the ant-excluded colonies and 11 (50%) of the ant-attended colonies lasted for more than 30 d (Fig. 1). Failure-time analysis showed that ant-excluded colonies were more likely to become extinct than those attended by ants (Log-rank test, d.f. = 1, $\chi^2 = 15.9$, $P < 0.0001$).

Species diversity of aphid predators was higher in the ant-excluded colonies than in ant-attended colonies (Table 1). The clubionid spider, *Clubiona* sp., was the most dominant predator in ant-excluded colonies, which were more frequently invaded by this spider than were the ant-attended colonies (Fisher's exact test, d.f. = 1, $\chi^2 = 19.3$, $P < 0.0001$), suggesting a positive effect of ant attendance on this predator. Another dominant predator, the larvae of *Episyrphus balteatus* de Geer, was found in both treatments, and there was no significant difference between treatment types regarding the proportion of colonies invaded (Fisher's exact test, d.f. = 1, $\chi^2 = 0.419$, $P = 0.517$). Our observation shows that the ants did not try to remove the larvae of *Episyrphus balteatus*. There was a great difference between the ant-excluded and ant-attended colonies regarding the proportion of colonies invaded by any predator (Fig. 2). Logistic regression revealed that the presence or absence of ant attendance had a significant influence on the frequency of colonies in-

Table 2. Logistic regression for the frequency of colonies invaded by predators in the ant-excluded treatment and in the ant-attended treatment.

Source	d.f.	χ^2	<i>P</i>
treatment	1	21.2	0.0000
days	1	0.546	0.4600

vaded (Table 2), but that census dates had no significant influence, implying that ant-excluded colonies suffered from predation throughout the study period (Fig. 2).

Effects of ant attendance in predator-free environments

MANOVA indicated that the presence or absence of ant attendance had a significant effect on the overall relationship among the aphids' morphological and reproductive traits (Table 3). In addition, a significant seasonal effect was detected by MANOVA (Table 3), suggesting that changes in the quality of phloem sap during this experiment affected the aphids' performance. The detection of significant difference between treatments allowed the application of randomized block ANOVA to each response variable. No significant effect was found in the interaction between season and treatment for any response variable (randomized block ANOVA, for body width, $F = 2.17$, $P = 0.148$, for hind femur length, $F = 1.35$, $P = 0.252$, for mature embryo number, $F = 1.22$, $P = 0.275$, and for total embryo number, $F = 0.88$, $P = 0.355$). Thus, in the randomized block ANOVAs, the interaction term was pooled with the error term (Table 4). The results of the ANOVAs indicated that the presence of the attending ants had significantly negative effects on the aphids' growth and fecundity: body width, hind femur length, and total embryo number were significantly larger in ant-excluded colonies than in ant-attended colonies (Table 4 and Fig. 3).

Discussion

The benefits of ant attendance to aphids have been widely appreciated (Way 1954, 1963, Tilles and Wood 1982, Bristow 1984, Cushman and Whitham 1989,

Völkl 1992, Stechmann et al. 1996). This study also confirmed attending ants' crucial effects on the persistence of aphid colonies. The attending ants in this system provided effective defenses for the aphid colonies against natural enemies. The absence of sooty mold was observed not only in ant-attended colonies but also in ant-excluded colonies, suggesting that it is not an important factor in the promotion of ant-aphid interaction.

In the experiments under predation, some aphids in ant-attended colonies might have dispersed more frequently than in ant-excluded colonies because of the lack of sticky barriers on the former colonies. This effect might have led to an overestimation of the colony extinction rate of ant-attended colonies. Nevertheless, the fact that the extinction rate of ant-excluded colonies exceeded that of ant-attended colonies clearly demonstrates that predation pressure is a critical factor mediating and intensifying the mutualism in this system. Observations of predators throughout the study period showed that attending ants effectively prevented *Clubiona* spiders in particular from invading the aphid colonies.

Aphids vary their behavior depending on whether they are attended by ants or not (Way 1963). Honeydew excretion rates, in particular, are much enhanced by the tactile stimuli of ants. In *Aphis craccivora* Koch, the excretion rate under ant attendance was seven times as high as that when the attending ants were removed (Takeda et al. 1982), and in *Aphis fabae*, it increased up to two-fold (Banks and Nixon 1958). These facts suggest that ant attendance promotes feeding activities and so increases excreting rates. Banks and Nixon (1958) experimentally confirmed an increase in the feeding rate of *Aphis fabae* under ant attendance by maintaining the aphids on broad beans that were cultivated in Hoagland's solution labelled by radioactive ^{32}P . They concluded that the aphid can alter its feeding activities by controlling the sucking pump in its head. Changes in excretion behavior according to whether attending ants are present or not are also observed in *T. quercicola* (Yao unpubl.). Interestingly, increased feeding rates under ant attendance do not lead to a proportional increase in reproductive rates per aphid (Banks and Nixon 1958, Takeda et al. 1982), although some reports claim that ant attendance somewhat enhances the fecundity of aphids (El-Ziady and Kennedy 1956, Banks

Table 3. MANOVA for effects of treatment (ant-attended and ant-excluded) on morphological and reproductive traits (body width, hind femur length, and mature and total embryo numbers). Pillai's trace statistic was used in calculating *F* statistic.

Source	Pillai's trace	<i>F</i>	Num. d.f.	Den. d.f.	<i>P</i>
Tree	0.4771	2.9764	8	76	0.0059
Branch (Tree)	1.6441	1.6420	68	160	0.0059
Treatment	0.2355	2.8489	4	37	0.0373
Season	0.6758	19.2835	4	37	<0.0001

Table 4. Randomized Block ANOVA for the effects of treatments (ant-attended and ant-excluded) on each trait. * indicates the significant difference between treatments or between seasons at a significance level of 0.05 after the application of the sequential bonferroni method.

	d.f.	SS	MS	F	P
(a) Body width					
Tree	2	0.041	0.020	7.13	0.0023
Branch (Tree)	17	0.132	0.008	2.71	0.0048
Treatment	1	0.031	0.031	10.90	0.0020*
Season	1	0.134	0.134	46.94	0.0001*
Error	40	0.114	0.003		
(b) Hind femur length					
Tree	2	0.001	0.000	0.65	0.5278
Branch (Tree)	17	0.034	0.002	2.95	0.0025
Treatment	1	0.005	0.005	6.75	0.0131*
Season	1	0.018	0.018	27.41	0.0001*
Error	40	0.027	0.001		
(c) No. mature embryos					
Tree	2	0.047	0.023	0.02	0.9848
Branch (Tree)	17	49.812	2.930	1.92	0.0458
Treatment	1	3.248	3.248	2.12	0.1529
Season	1	17.640	17.640	11.53	0.0016*
Error	40	61.177	1.529		
(d) Total no. embryos					
Tree	2	19.347	9.673	1.89	0.1641
Branch (Tree)	17	282.807	16.636	3.25	0.0011
Treatment	1	37.053	37.053	7.25	0.0103*
Season	1	89.234	89.234	17.45	0.0002*
Error	40	204.555	5.114		

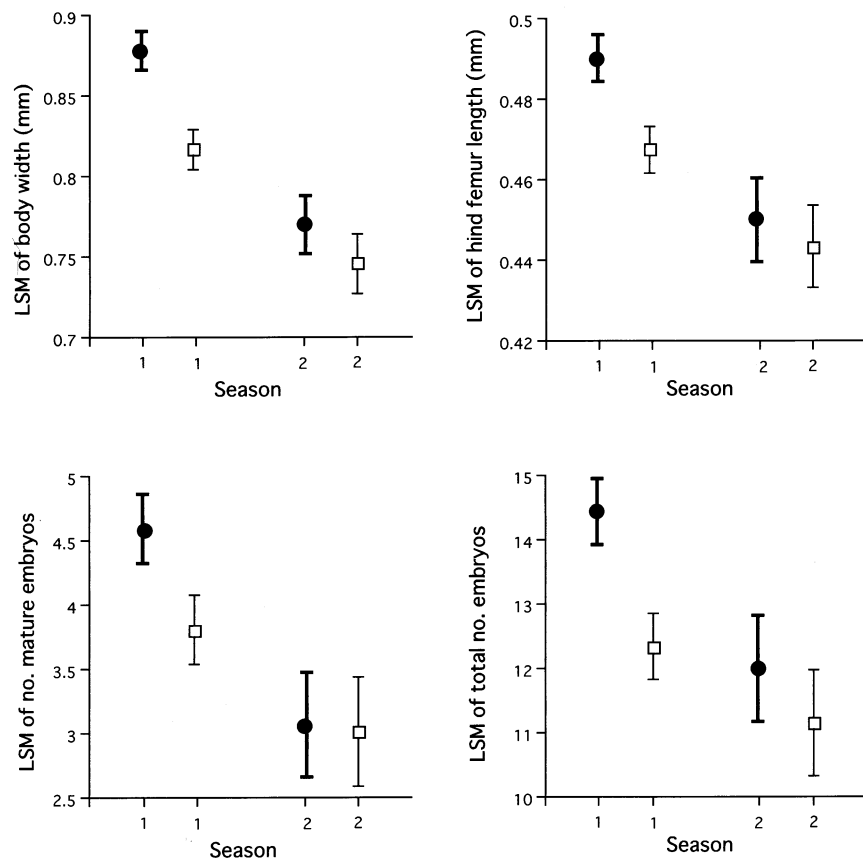
1958, El-Ziady 1960). In contrast, Stadler and Dixon (1998) demonstrated that the fecundity of aphids was significantly lower when they were attended by ants than when they were not. The fact that an increase in feeding or excreting rate does not lead to a corresponding increase in reproductive rate can be explained by assuming that the faster rate of nutrient absorption by aphids under ant attendance allows the aphids in turn to offer greater amounts of honeydew to visiting ants as rewards to maintain their continuous visitation. It has been reported that sugars present in honeydew are not the same as those contained in the phloem sap. Phloem sap obtained from the cut stylets of *Tuberolachnus salignus* (Gmelin), which is dwelling on willow leaves, contained sucrose alone, whereas the honeydew is composed of roughly equal amounts of sucrose, glucose, fructose, and melezitose (Mittler 1958). Michel (1942) and Maurizio (1985) found that among these sugars, melezitose was conspicuously abundant in aphid honeydew. Similarly, *T. quercicola* produces honeydew that is abundant in melezitose (Yao unpubl.). Völkl et al. (1999) pointed out that melezitose should play a critical role in ant-aphid relationships because melezitose is most preferred by ants. Furthermore, Kiss (1981) proposed the hypothesis that aphids synthesize melezitose in particular in order to attract attending ants. We suggest that such an aphid response to ants has evolved as a result of the exploitation of aphids by the visiting ants.

One reason for the difficulty in detecting the costs of ant attendance in aphids may be that the experimental

removal of the attending ants results in an unusual condition for aphids, particularly for those attended obligatorily by ants. If aphids have fully achieved adaptation to ant attendance, the removal of the ants may incite the aphids' impulse to escape, which may result in a decrease in feeding rates and the consequent decline in reproductive rates. Thus, reduced fecundity due to the removal of attending ants may not indicate that ant attendance has positive effects on aphid reproduction. In *T. quercicola*, it was not observed that the exclusion of attending ants triggered escape behavior.

The body size and fecundity of aphids in both types of treatment decreased from early summer to autumn (Fig. 3). This tendency is most probably due to seasonal changes in the quality of their host plant. When trees are actively growing or senescing, the phloem sap contains relatively high concentrations of amino-nitrogen composed of many amino acids, but in summer the sap is low in amino-nitrogen and contains relatively few amino acids (Mittler 1958). Accordingly, aphid body size and per capita reproductive rate peak in spring when leaves are growing and decline after midsummer (Dixon 1970). Additionally, during midsummer, the level of tannins rapidly increases in leaves of the *Quercus* species (Kudo 1996, Murakami 1998), and tannins clearly lower insect performance (Feeny 1970, Karowe 1989). Whether the costs of ant attendance can be detected may depend on the quality and quantity of resources available to the aphids. When food resources are abundant, the aphids could allocate large amounts of resources to the honeydew when they are requested

Fig. 3. Least squares means for aphid body size (body width and hind femur length) and numbers of mature and all embryos. Least squares mean \pm SE for two seasons (season 1, from 23 July to 11 August; season 2, from 12 August to 31 August). Closed circles, ant-excluded treatment; Open squares, ant-attended treatment.



to do so by the ants. As a result, ant attendance may lead to a large reduction in fecundity. When resources are declining, however, the aphids would be unable to afford to allocate sufficient amounts of nutrients to the honeydew, levelling out the difference in fecundity between the treatments.

It has been documented that when honeydew production by the aphids begins to decline, ants sometimes resort to hunting the aphids which they have been attending (Edinger 1985, Sakata 1995). Furthermore, because aphid colonies producing less honeydew attract fewer ants, such colonies are vulnerable to natural enemies. It is therefore likely that the excretion rate of honeydew is a trait that is subject to selective pressures via the honeydew-collecting behavior of ants. If variance existed among aphid clones in the quality and quantity of the honeydew excreted in response to the demands of ants, those clones that produced more and better honeydew would be favored. Once aphids had gained benefits from ant attendance, the attending ants may have functioned as a selective agent that has strengthened and promoted aphids' excreting behavior. Evaluation of the hypothesis that manipulation and exploitation by ants have led to the reinforcement of the honeydew-excreting behavior in aphids would

provide a profound understanding of the evolution of ant-aphid interactions.

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