Ant–aphid mutualism: the influence of ants on the aphid summer cycle

Karolina Tegelaar, Mattias Hagman, Robert Glinwood, Jan Pettersson and Olof Leimar


There are few longtime studies on the effects on aphids of being tended by ants. The aim of this study is to investigate how the presence of ants influences settling decisions by colonizing aphids and the post-settlement growth and survival of aphid colonies. We conducted a field experiment using the facultative myrmecophile Aphis fabae and the ant Lasius niger. The experiment relied on natural aphid colonization of potted plants of scentless mayweed Tripleurospermum perforatum placed outdoors. Ants occurred naturally at the field site and had access to half of the pots and were prevented from accessing the remainder. The presence of winged, dispersing aphids, the growth and survival of establishing aphid colonies, and the presence of parasitoids were measured in relation to presence or absence of ants, over a period of five weeks. The presence of ants did not significantly influence the pattern of initial host plant colonization or the initial colony growth, but ant-tended aphids were subject to higher parasitism by hymenopteran parasitoids. The net result over the experimental period was that the presence of ants decreased aphid colony productivity, measured as the number of winged summer migrants produced from the colonized host plants. This implies that aphids do not always benefit from the presence of ants, but under some conditions rather pay a cost in the form of reduced dispersal.

Interspecific mutualism is defined as an interaction between species that produces net benefits for the participants, and it is both ubiquitous and ecologically important in natural ecosystems (Axelrod and Hamilton 1981, Boucher et al. 1982). The costs associated with utilizing the services provided by mutualistic partner species should on average be lower than the benefits gained, but shifting circumstances can influence the balance between costs and benefits and this context dependence can make it difficult to interpret the interaction as truly mutualistic (Bronstein 1994, 2001a, b). Here we address this issue for an aphid–ant mutualism.

Aphid feeding on phloem sap results in honeydew, a sugar-rich byproduct that is utilized by many organisms (Völkli et al. 1999). The aphid–ant interaction is commonly described as an exchange of favors in which honeydew is exchanged for protection from predators and parasitoids (Stadler and Dixon 2005). In earlier studies honeydew excretion was reported not to incur costs for the tended aphids (El Ziad and Kennedy 1956, Banks 1958, El Ziad 1960). Among the observed benefits for myrmecophiles of ant tending are lower mortality risks from predation and parasitism (Pierce and Mead 1981, Völkli 1992, Takizawa and Yasuda 2006), an increased reproductive rate (Flatt and Weisser 2000), enhanced rates of feeding and excretion (Banks 1958) and more rapid development and colony growth (El Ziad and Kennedy 1956, El Ziad 1969). Ants are also known to clean honeydew residue from host plants, reducing pathogen incidence and promoting a healthy aphid colony and host plant (Way 1963).

However, the evidence for aphid benefits is equivocal. For example, aphids can alter the honeydew composition, presumably to make it more attractive to ants, and this might involve costs (Yao et al. 2000, Yao and Akimoto 2001, 2002). This appears consistent with the observation by Stadler and Dixon (1998) that tended aphids developed more slowly, were smaller in size, and invested less in reproduction. Further, the larger colony size observed for tended aphids need not be attributed to an increased reproductive rate, as previously reported, but could be a consequence of ant manipulation of aphid dispersal. Ant derived substances have been shown to reduce the production of winged summer migrants (Kleinjan and Mittler 1975, El-Ziady and Kennedy 1956, El-Ziady 1960), delay dispersal (Kindlmann et al. 2007), and reduce the mobility of non-winged individuals (Oliver et al. 2007). These ant-mediated effects could increase aphid colony size, but might not be beneficial for the aphids.

Multiple species interactions present further complications for the understanding of mutualism, as more than two species with conflicting interests are present simultaneously. Costs and benefits for aphids in terms of ant protection against their natural enemies are often not discussed in relation to particular predators or parasitoids, although there may be important differences between them as aphid behavior and reaction to threat might differ depending on
the type of enemy. For example, in addition to the possible ant–aphid mutualism, a conflicting ant–parasitoid mutualism has been discovered (Völk 1992, Kaneko 2003, Mondor et al. 2008). Some hymenopteran parasitoid species prefer to attack homopterans tended by ants because the ants protect the parasitoid offspring against hyperparasitoids and predators (Völk 1992, Mackauer and Völk 1993, Novak 1994). Völk (1992) showed that aphids that have been attacked by certain parasitoids produce more honeydew than unparasitized individuals, thereby creating the possibility of a conflicting mutualism. Because aphids have different kinds of enemies, like parasitoids, predatory lacewings and ladybirds, the effects of ant attendance will depend on the particular enemies that are present in a locality, and this could be quite variable.

There are also likely to be differences in the need for protection in different phases of aphid colony growth. Addicott (1978) showed that aphid colonies in the initial growth phase had better survival if tended by ants, whereas for larger colonies the effect was opposite or neutral. Because early ant–aphid contact may involve an increased risk of ant attack (Glinwood et al. 2003), there may be reasons for aphids to invest more in the interaction in the initial phase, whereas the value of ants for the aphids might be lower or negative in later phases.

To establish whether ants are beneficial or costly for aphids, one needs to assess the overall influence of ant attendance on the productivity of critical phases of the aphid life cycle such as the parthenogenetic summer cycle. All extant aphids reproduce parthenogenetically (Moran 1992), often by cyclic parthenogenesis, which combines asexual reproduction during the summer cycle with sexual reproduction in the autumn (Dixon 1998, Simon et al. 2002). Aphid morphs connected to aphid life cycles include among others the founder of the clone (fundatrix), migrants, parthenogenetic wingless multipliers and sexuals depending on the aphid species (Dixon 1998). Both winged (alate) and wingless (apterous) females are present in the parthenogenic phase, depending on colony conditions (Moran 1992, Dixon 1998).

A relevant measure of the influence of ant tending on aphid productivity might then be the effect of ants on the number of winged emigrants produced during the lifetime of an aphid colony that was founded by an incoming winged migrant landing on a host plant. Such a measure would take into account several kinds of ant-mediated effects, both positive and negative, including aphid colony growth, survival and the extent and timing of winged emigrant production. In a field-experiment with naturally occurring aphids and ant populations, we attempted to measure the influence of ant attendance on productivity of the aphid summer cycle, identifying factors that may determine the overall consequences of ant attendance for aphids.

Methods

Study species

We investigated interactions between the aphid *Aphis fabae* (Homoptera: Aphididae) and the ant *Lasius niger* (Formicidae). *Lasius niger* commonly tend several species of aphids in the field, including *A. fabae* which is a facultative ant mutualist and is frequently used in studies of ant-aphid mutualism (El Ziady and Kennedy 1956, Banks 1958, Stadler and Dixon 1998). *Aphis fabae* overwinters as eggs on spindles *Euconymus europaeus*, guelder rose *Viburnum opulus*, or sweet mock-orange *Philadelphus coronarius*. Eggs hatch in early spring and colonies develop on young leaves. Winged migrants are produced in June, and fly to a wide range of summer host plants, including scentless mayweed *Tripleurospermum perforatum* (Blackman and Eastop 2000). Populations develop on the summer hosts, reaching a peak during July, at which time they either change host plant, often to spear thistle *Cirsium vulgare*, or develop the first winged autumn migrants that fly back to the winter host (Dixon 1998).

Experimental setup

The experiment was conducted in a field 6 km southwest of Uppsala, at the Swedish Univ. of Agricultural Sciences, from 17 June to 15 July 2008. The field and the bordering areas contained several large naturally occurring colonies of *L. niger* and had a high density of *T. perforatum*. We transferred naturally occurring plants of *T. perforatum* (10–15 cm tall) to pots of equal sizes (20 cm ø and 25 cm high) containing soil taken from the study site. A total of 96 pots of *T. perforatum* were numbered and placed in six treatment patches with 16 pots in each patch, placed in a 4 × 4 grid with 0.5 m spacing between pots. The six patches were positioned along one of the edges of the field, with 1.5 m from the edge of one patch to the start of the next. In every second patch, a stripe of Tanglefoot was applied to the upper part of the pots and a fine mesh was glued to cover the drainage holes in the bottom of the pot, preventing ant from accessing these plants. Plants were watered once a week. The patches were located in the vicinity of *L. niger* colonies, allowing a natural level of ant attendance, and *L. niger* was the only ant species recorded on the host plants during the experiment. The plants were placed in the experimental arrangement for aphids to colonize during four days before the first data collection.

A suction trap for airborne aphids (at 12 m altitude), operated by the Swedish Univ. of Agricultural Sciences, was located nearby the experimental area and provided data on the timing of flying *A. fabae* during the study. The trap is part of a network of traps in different countries, which has been used for many years to study the timing of aphid migrations flights. Based on this experience, the traps are known to give reliable information.

Data were collected weekly between 09:00 and 13:00 over five weeks. The data included number of alates, estimated number of adult aphids, number of aphid clusters, numbers of ants on host plants and number of parasitoids. The number of aphids on a plant was estimated visually and classified as 0, 1–5, 6–10, 11–20, 21–30, 31–100, 101–200. In the fifth observation session the percentage parasitized aphids on each plant was recorded; these were easily recognized as mummified brown aphids. Data collection ended at this time because several aphid colonies had succumbed to parasitoid attacks and because the date corresponded to the
typical time for *A. fabae* to make a host-plant switch to spear thistle *C. vulgare*.

**Statistical analyses**

The data on aphid colony productivity as a function of ant treatment and week were analyzed with Bayesian methods: linear mixed models were fitted using the MCMCglmm function (ver. 2.12; Hadfield 2010) in the R statistics package (ver. 2.12.1; R Development Core Team 2010). The R package was also used for all other statistical tests.

The response variables were first transformed to achieve homogeneous residual variances, as judged by fitting standard linear models: the transformation \(\log(x + 0.2)\) was used for the number of alates per plant, \(\log(x + 0.5)\) for the number of aphid clusters per plant, and \(\log(x + 2)\) for the estimated number of aphids per plant. In the fixed effect part of the mixed model, we allowed for effects of observation session and ant treatment, and for differences in the effect of the ant treatment between sessions. As random effects we included treatment patch, plant nested in treatment patch, and a treatment patch by observation session interaction (the latter effect allows for the possibility that the patch effects differ between observation sessions). These random effects were supposed to model variation in local conditions (e.g. ant availability) among patches and plants.

Our reason for using a Bayesian Markov chain Monte Carlo method is that this is a reasonable approach to assess the statistical significance of fixed-effect terms in models with random effects. For this purpose we report the MCMC p-values provided by the MCMCglmm function for the different terms of the fixed effect part of the model (Table 1).

Table 1. Bayesian statistical analysis of the effect of time (observation session) and ant treatment on the number of alates, aphid clusters and estimated number of aphids per plant. Mixed effect models were fitted, with observation session, ant treatment and their interaction as fixed effects, and patch, plant nested in patch, and a patch by session interaction as random effects.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Term$^*$</th>
<th>(p_{\text{MCMC}})</th>
<th>Term$^*$</th>
<th>(p_{\text{MCMC}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alates per plant</td>
<td>Treatm1</td>
<td>0.563</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Session2</td>
<td>0.241</td>
<td></td>
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<tr>
<td></td>
<td>Session3</td>
<td>0.197</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session4</td>
<td>0.870</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session5</td>
<td>0.994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clusters per plant</td>
<td>Treatm1</td>
<td>0.626</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Session2</td>
<td>0.041</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Session3</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Session4</td>
<td>0.001</td>
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<td></td>
<td>Session5</td>
<td>0.001</td>
<td></td>
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</tr>
<tr>
<td>Aphids per plant</td>
<td>Treatm1</td>
<td>0.656</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Session2</td>
<td>0.060</td>
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<td></td>
<td>Session3</td>
<td>0.001</td>
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<td></td>
<td>Session4</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Session5</td>
<td>0.001</td>
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</tbody>
</table>

$^*$see methods section for description of the Bayesian MCMC analysis. The terms correspond to the so-called ‘treatment contrasts’ for the fixed-effect part of the model: Treatm1 is the difference between ant excluded and ant attended plants in the first session, Sessionk is the difference between session k and session 1 for ant attended plants, and Sessionk \(\times\) Treatm is the difference between session k and session 1 of the difference between ant excluded and ant attended plants.

In the MCMC analyses, we used 10 000 burn-in iterations, followed by 250 000 iterations sampled with a thinning interval of 25, resulting in a sample size from the posterior distribution of 10 000. The variance components of the random effects were given inverse-Wishart prior distributions with variance parameters such that the total observed variance in the response variable was split evenly between the residual and the random effects, and for the random effects the prior gave equal weight to each of them.

**Results**

The ant exclusion treatment was successful, in the sense that very few ants were found on the plants in the pots with a Tanglefoot barrier. Occasionally a few ants could still access these pots, for instance by climbing on temporary grass bridges, but were considerably fewer than on the plants in the pots with free ant access (Fig. 1). Low numbers of ants were recorded in the first observation session; most plants with ant access were without ants at this time. The number of ants per plant peaked in the third observation session, followed by a slight decline in the following two sessions (Fig. 1).

An important measure of the productivity of aphid colonies in the experimental situation is the number of alates (winged emigrants) produced from the offspring of an incoming alate settling on a host plant. There was no significant difference in the number of alates present on host plants in the first session (Treatm1 term in Table 1, Fig. 2), suggesting that the number of incoming alates were similar between treatments. On the other hand, the statistical significance of the Session 4 \(\times\) Treatm and Session 5 \(\times\) Treatm terms in Table 1 indicates that the difference in the number of alates between unattended and attended plants was greater in session 4 and 5 than in the first session (Fig. 2). Thus, there were fewer alates on plants with ant access in the later part of the experiment. These alates were likely, at least in part, to have been born on the plants they were found on, indicating decreased aphid productivity on plants with ant attendance. A similar pattern was seen for both the number

![Figure 1. Number of ants present on plants during the experiment.](image-url)
of aphid clusters per plant (Fig. 3a) and the estimated number of aphids per plant (Fig. 3b), with statistically significant Session 5 × Treatment terms (Table 1). In the final observation session, the number of aphid clusters and the number of aphids per plant were lower for plants with ant access (Fig. 3). Comparing our observations of alates on the host plants with the alates captured in the nearby suction trap (Table 2), we see that our experiment coincided with the timing of the natural aphid settlement in the area.

Aphids tended by ants had a higher risk of parasitoid attack, as indicated by a higher overall number of parasitoids observed on plants with ant access (Fig. 4a) and a statistically significantly higher proportion of parasitized aphids (mummies) on day 33 (Welch two-sample t-test of the average percentage mummies on plants between patches with and without ant access: \( t = 5.75,\) \( DF = 2.215,\) \( p = 0.023 \)). The percentages of mummified aphids per plant (mean ± SE) in the final observation session was (20.92 ± 4.6) and (0.938 ± 0.7) for plants with and without ant access respectively. The number of parasitoids per plant had a skewed distribution, with many plants having no parasitoids, making statistical testing at the plant level difficult. By averaging over the plants in a patch, and also averaging over observation session 3–5, we found a statistically significant positive correlation between the average number of parasitoids and the number of ants per plant (\( r = 0.944,\) \( t = 5.749,\) \( DF = 4,\) \( p = 0.005 \); Fig. 4b), supporting the conclusion that ant attendance increased the risk of parasitoid attack.

**Discussion**

Host alternating aphids such as *Aphis fabae* hatch on the winter host plant and produce winged offspring (alates) that eventually migrate and colonize summer hosts (Dixon 1998). During the summer, a sequence of several hosts can be exploited through colonization by incoming migrants, followed by the growth of a colony of wingless multipliers on the host and the subsequent production of winged emigrants. A large production of alates is important for the success of a parthenogenetic colony. They can disperse to new host plants when the quality of the current one declines (Mittler and Sutherland 1969), when increasing colony size impedes further growth (Johnson 1965) or in response to the presence of natural enemies (Dixon and Agarwala 1999). The alate aphids are the clone’s means of dispersal and are crucial for its reproductive success.

It follows that the output of emigrating alates per colonizing alate is a useful measure of the productivity of the summer phase of the aphid life cycle. Applying this measure to our field experiment indicates that ant access led to a decrease in aphid productivity. We found no statistically significant difference between treatments in the number of alates per plant in the first observation session (Fig. 2) and

![Figure 2](image2.png)

**Figure 2.** The number of alate (winged) aphids per plant (mean ± SE) on attended and unattended plants during the experiment. There were 96 plants, 48 in each of the two treatments.

![Figure 3](image3.png)

**Figure 3.** Number of aphid clusters (a) and the estimated number of aphids (b) on attended and unattended plants during the experiment (mean ± SE). A cluster is defined as a separate group of aphids living on the host plant. Aphid numbers in (b) are approximate estimates. There were 96 plants, 48 in each of the two treatments.

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<table>
<thead>
<tr>
<th>Week of experiment</th>
<th>Pre 2</th>
<th>Pre 1</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of <em>A. fabae</em></td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

**Table 2.** The number of *A. fabae* caught in a suction-trap at the site during each week (1–5) of the experiment and the number of captured aphids one (Pre 1) and two weeks (Pre 2) prior the onset of the experiment. No *A. fabae* were collected at the site earlier than Pre 2.

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spring against hyperparasitoids and predators. Because sitoid preference is that ant tending protects the parasitoid’s 1999, Mondor et al. 2008). A possible reason for this para-

attacks, and it is unlikely that they would survive longer than the less parasitized colonies on plants without ants.

Aphid–ant interactions are often described as mutually beneficial exchanges in which honeydew is traded for protec-
tion from predators and parasitoids (Stadler and Dixon 2005). Our results indicate that aphids sometimes instead vary between different aphid–ant interactions, for instance because parasitoid and predator faunas vary in space and time. Such conditionality in the outcome of seemingly mutualistic interactions could well be widespread. Further, since mutualism is a common phenomenon, it would not be surprising if different mutualisms occasionally interfere with one another. Thus, in light of our results, the answer to the question posed by Völk (1992), whether aphids or their parasitoids benefit from ant attendance, seems to be that it is the parasitoids.

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