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Role of ants in structuring the aphid community on apple

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Abstract. 1. The aphids *Dysaphis plantaginea* Passerini, *Aphis* spp. (*Aphis pomi* De Geer and *Aphis spiraecola* Patch), and *Eriosoma lanigerum* Hausmann are commonly found together in apple orchards. Ants establish a mutualistic relationship with the myrmecophilous aphids *D. plantaginea* and *Aphis* spp. but not with *E. lanigerum*.

2. Field surveys and one experiment manipulating the presence of ants and the aphid species were conducted to test the hypothesis that ants play a role in structuring the community of these aphids on apple.

3. Ants tended *D. plantaginea* and *Aphis* spp. but not *E. lanigerum* colonies. In the field, *D. plantaginea* performed better in the presence of ants while no effect was observed in *Aphis* spp. Contrarily, populations of *Aphis* spp. in the manipulative experiment performed better in the presence of ants while no differences were observed for *D. plantaginea*. Such differences between field and manipulative conditions could be related to thermal tolerance, phenology, and life cycles. In contrast, populations of *E. lanigerum* were reduced in the presence of ants.

4. Ants also had a significant negative effect on the abundance of natural enemies, which could partially explain the benefits to the tended aphids. However, while ants did not provide a benefit to *Aphis* spp. when it was reared alone, in the presence of other species ant attendance increased *Aphis* abundance by 256% and simultaneously reduced *E. lanigerum* abundance by 63%. Therefore, ants benefited *Aphis* by reducing competition with other aphid species, which involves a different mechanism, explaining the benefit of ant attendance. Considering all the aphid species together, ants had a net positive effect on aphid abundance, which was consequently considered harmful for the plant.

5. Our results highlighted the role that ants play in structuring apple aphid communities and give support to the observed pattern that ants can benefit tended aphids while simultaneously reducing the abundance of untended herbivores.

Key words. ant–aphid mutualism, *Aphis*, coexistence, community ecology, competition, *Dysaphis plantaginea*, *Eriosoma lanigerum*, indirect effects, predators.

Introduction

The mutualisms between ants and some aphid species have received much attention (e.g. Banks, 1962; Bishop & Bristow, 2003; Mooney, 2006; Nagy *et al*., 2007; Smith *et al*., 2008; Stewart-Jones *et al*., 2008). In such mutualisms, aphids typically provide honeydew for ants while ants provide enemy-free space for aphids. Many studies have shown the increase of aphid populations mediated by the negative effect of ants on predators and parasitoids (e.g. Banks, 1962; Bishop & Bristow, 2003; Nagy *et al*., 2007; Stewart-Jones *et al*., 2008). In fact, the ant-mediated reduction of the populations of natural enemies of aphids has been proposed as the primary mechanism explaining how aphids benefit from the ant–aphid mutualism.

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Much less studied, aphid–ant mutualisms can also have ecological consequences for other community members living in the same host (Crutsinger & Sanders, 2005; Mooney, 2006; Savage & Peterson, 2007; Styrsky & Eubanks, 2007; Smith *et al*., 2008). A recent review on the role of interactions between ants and honeydew-producing hemipterans showed that ants have mostly negative effects on the abundance and richness of other herbivores sharing the same host (Styrsky & Eubanks, 2007). Particularly in aphid communities, ants have been shown to have a determinant influence in promoting aphid coexistence on common milkweed *Asclepias syriaca* (Smith *et al*., 2008). Promoting the fitness of the tended aphid, ants allowed the coexistence with two other competitively superior aphids that were non-tended. Thus, the reduction of interspecific competition could be seen as another mechanism explaining how ants are beneficial to the tended aphids. In addition, through different interactions with each aphid species, ants may play an important role in structuring aphid communities (Smith *et al*., 2008).

In the context of these complex and contrasting interactions with different herbivores, ants may also have ecological consequences for the host plant. On the one hand, plants suffer the damage of the increasing populations of the tended aphids. On the other hand, plants take advantage of the ant-mediated reduction of other herbivores. Thus, the balance between the cost of the feeding of the tended aphids and the benefit of the ant-mediated elimination of some other herbivores will determine the effect, negative or positive, of ants on plants. In 22 out of 30 studies reviewed by Styrsky and Eubanks (2007), ant–hemipteran interactions benefited plants by ant removal or harassment of non-tended, more damaging, arthropods. Another recent review (Rosumek *et al*., 2009) also highlighted the importance of ants as plant biotic defences: plants without ants suffered almost twice as much herbivory than plants with ants.

In Asturias (north-west Spain) apple trees are usually colonised by three specialist aphids: the rosy apple aphid (*Dysaphis plantaginea* Passerini), the green apple aphid (*Aphis pomi* De Geer), and the woolly apple aphid (*Eriosoma lanigerum* Hausmann). As these aphid species frequently co-occur on the same tree, there could be some degree of interspecific competition among them. These species may partition host resources and avoid competition by feeding on different plant structures. In fact, *D. plantaginea* and *A. pomi* feed on growing shoots while *E. lanigerum* feeds on harder parts of the tree. Nevertheless, despite the fact that the feeding positions on the tree may not overlap, the aphids are feeding on a common resource: phloem sap (Dixon, 1998). In such a situation, different factors might influence the structure of the aphid community and ants might participate in shaping the aphid community (Mooney, 2006; Smith *et al*., 2008). *Dysaphis plantaginea* as well as *A. pomi* are frequently ant-tended (Nagy *et al*., 2007; Stewart-Jones *et al*., 2008). Both are relatively inconspicuous, grey and green coloured, respectively. On the contrary, *E. lanigerum* is a non-myrmecophilous aphid covered by a wax mass, which is thought to have a protective role against natural enemies (Moss *et al*., 2006).

In our research, we aimed to determine (i) the effect of ants on the population development of *D. plantaginea*, *Aphis* spp., and *E. lanigerum* and on their natural enemies, (ii) the mechanisms involved in such effects, (iii) the balance between the negative and positive effects of ants on non- and mutualist apple aphids, and (iv) the role that ants play in structuring the community of these aphids on apple.

Materials and methods

Field observations were carried out in two organic apple orchards to assess the influence of ant attendance on the natural development of aphid colonies. An additional experiment was conducted to assess the effect of tending in structuring apple aphid community. All the experiments were carried out at Asturias, Spain, in spring and summer 2008.

Aphid life cycles

Dysaphis plantaginea is a host-alternating species that hatches from overwintering eggs in early spring and develops for several generations on apple before the obligatory migration to the secondary host, *Plantago* spp. In autumn, this aphid returns to apple trees where sexual reproduction and oviposition take place. *Aphis pomi* completes its whole life cycle on apple. In a survey during the experiment, *A. pomi* and the spirea aphid, *A. spiraecola* Patch, were found together in our apple orchards, as happens in North America (Fréchette *et al*., 2008) and some European countries (Andreev *et al*., 2007). These two species are very similar and hardly distinguishable in the field, so they will be referred to hereafter as green aphids *Aphis* spp. *Eriosoma lanigerum* develops anholocyclic populations in European apple orchards due to the lack of its primary host (*Ulmus americana* L.) (Marchal, 1928).

Field experiments

Orchard 1. This experiment was carried out in a 0.8 ha apple orchard planted in Sariego (43°24′N, 5°32′W) in 1998 with five different local cider-apple cultivars growing on MM.106 rootstocks. The assay was conducted on the cultivar ‘Durona de Tresali’, which is susceptible to *D. plantaginea*, *E. lanigerum*, and *Aphis* spp.

Dysaphis plantaginea is usually the first aphid colonising apple shoots in spring in Asturias. In early April, when the first fundatrices were observed, 20 trees of homogeneous size were selected. Then, 10 shoots with *D. plantaginea* fundatrices were randomly selected in each tree and marked with coloured plastic strips. Ten of those trees were randomly selected for the ant exclusion treatment. Ants were excluded with sticky paste (Tanglefoot Company, Grand Rapids, Michigan) applied on a band of adhesive tape (10 cm width) placed around the tree trunk approximately 1 m above ground. There were no overlapping branches that could facilitate ant movement among trees. Any ant observed in the exclusion treatment during sampling was eliminated.

From mid-April to the moment when all *D. plantaginea* disappeared, the 200 selected shoots were examined on a weekly basis for the presence of aphids, ants and natural enemies. Although the shoots were marked for the presence of *D. plantaginea*, the occurrence of *Aphis* spp. or *E. lanigerum* was also recorded.

Orchard 2. The experiment was carried out in a 0.4-ha orchard planted in Llanera (43°26′N, 5°47′W) in 1992 with two local dessert-apple cultivars grafted on M.9 rootstocks. The experiment was conducted on the local cultivar 'Reineta Encarnada', which is also susceptible to *D. plantaginea*, *E. lanigerum*, and *Aphis* spp. Sticky barriers on the trunk were not a good method for avoiding ants in this orchard due to strong branch overlapping among trees and the possibility of ant movements between adjacent trees. Therefore, 50 growing shoots with established *D. plantaginea* colonies were selected in mid-May, preferably no more than one shoot per tree. Twenty-five out of the 50 selected shoots were randomly assigned to the ant-exclusion treatment. To avoid the presence of ants in these shoots, sticky paste was applied to the shoot base. From mid-May to the moment when all aphids disappeared, the 50 selected shoots were examined on a weekly basis for the presence of aphids, ants and natural enemies.

No pesticides were sprayed just before or during the assay in these orchards.

To determine the ant species tending apple aphids, samples of ants from *D. plantaginea* and *Aphis* spp. colonies were collected not only from the experimental orchards, but also from apple trees in orchards and gardens along the regional area. From 73 field-collected samples, six and five species of ants were recorded tending *D. plantaginea* and *Aphis* spp., respectively. *Lasius niger* (L.) was the most abundant species (64%), followed by *Formica cunicularia* Latreille (18%), *L. emarginatus* (Olivier) (6%), *L. grandis* Forel (4%), *Myrmica rubra* (L.) (4%), *F. pratensis* Retzius (3%), and *F. fusca* (L.) (1%). Occasionally, more than one ant species were found tending the same aphid colony.

Manipulative experiment

This assay was carried out on apple plants to test the role of ants in structuring the apple aphid community following the methodology of Smith *et al.* (2008). For that, plants were infested with each species (*D. plantaginea*, *Aphis* spp., and *E. lanigerum*) alone and with all three aphids together. Then, ants were allowed to tend aphids in half of the plants. Research was conducted from the end of June to the end of July 2008 at SERIDA (Institute of Research on Food and Agriculture of Asturias) located in Villaviciosa (43°28′N, 5°26′W). The experiment was carried out on the local cider cultivar 'De la Riega', which is susceptible to all these aphids. This cultivar was grafted on MM.106 rootstocks and planted in 4-litre pots.

Experimental approach. Aphid response was compared in the presence and absence of ants. The aphid treatment included each species alone and all three together. Pairwise coexistence treatments were not included because the required sample size was logistically prohibitive. The number of each aphid species in the multiple species treatment was one-third of that in the single species treatment, to maintain the initial aphid density constant among the four treatment levels. The four aphid treatments (each species alone and all three together) and the two ant treatments (exclusion and control) were factorially combined with 13 replicates for a total of 104 plants. Ants were excluded in half of the plants of each aphid treatment by placing sticky paste around the pots.

When plants were about 25 cm in height, infestation was performed inside a greenhouse. Six apterous aphids (six and two per species in single- and mixed-species treatment, respectively) were carefully placed with a small paintbrush on the youngest leaf of each plant. Aphids used for infestation derived from field-collected individuals were reared separately on susceptible apple plants. Reinfestation was performed when necessary, 1 and 3 days after initial infestation to complete the number of six aphids per plant.

Four days after infestation, plants were taken outside to allow interactions with ants. Plants were randomly distributed on five 1-m high tables to facilitate observations and sampling. Since no ant attendance occurred naturally, two ant nests (found inside flowerpots) were placed on each table. Sticks were placed between these ant nests and the apple plants of the control treatment to facilitate ants to reach aphid colonies. This method was very successful since all the control plants were regularly visited by ants. All the ant nests belonged to the species *Lasius niger* (L.).

As the experiment took place near apple orchards in July, when dispersion of *Aphis* spp. is important, all the experimental plants suffered to some extent colonisation by winged *Aphis* spp. migrants. This fact provided us with a more realistic situation, closer to what really happens in the field. However, this was a handicap for sampling and handling data.

Ants, aphids, and natural enemies per plant were counted 8, 14, 25, and 34 days after infestation.

Level of ant attendance and ant behaviour. The level of ant attendance was assessed by counting the number of ants per plant. Interactions between ants and aphids were characterised by observing ant behaviour in each aphid treatment. For that, an ant was randomly selected and observed during 5 min or until the ant left the plant. We recorded the time spent in each of these behaviours: aphid tending, roaming, predation, and others (such as ant interactions or being motionless). From 17 to 21 observations were made in each aphid treatment.

Aphid abundance. Aphid abundance on each leaf was described by six values: 0, no aphids; 1, 1–5 aphids; 2, 6–25 aphids; 3, 26–125 aphids; 4, 126–625 aphids; and 5, 626–3125 aphids. For the analysis, these values were reconverted to aphid numbers substituting each value for the central value of the abundance class represented: 0 was not substituted; 1 was substituted by 3; 2 by 15.5; 3 by 75.5; 4 by 375.5, and 5 by 1875.5. The total aphid number per plant was then calculated as the sum of the values on each leaf. Since the total counts of *Aphis* spp. in the *Aphis* and in the multiple species treatments were due to populations derived from both the initial infestation and the arrival of migrants, we removed the migrant effect by subtracting the estimated number of migrants from the total *Aphis* counts in those plants. The number of migrants was estimated as the mean number of *Aphis* counted on plants initially infested only with *D. plantaginea* or *E. lanigerum*.

Data were transformed to per capita progeny production dividing final aphid density by the initial aphid density per plant (two or six in the single and the multiple species treatment, respectively). As there were few or no aphids at some controls, we averaged aphid counts of the four control dates to improve distribution and reach the statistical assumptions of the tests employed.

Data analysis

Field data were analysed independently for each orchard. Data from the first sampling dates were not used in the analyses until ants were observed for the first time and consequently an ant-tending effect could be expected. The effect of ants on the percentage of shoots infested with aphids was analysed using Wilcoxon's signed-rank tests. The influence of ant attendance on aphid number was analysed using a repeated measures analysis of variance (ANOVAR) after a $\log_{10}(x+1)$ transformation of aphid number before analysis, to meet assumptions of normality. Spearman correlations coefficients were calculated to test the existence of statistical correlation between colony size and the number of ants per colony. For natural enemy differences in abundance between treatments, χ^2-tests were performed on the total number of natural enemies and on the ratio aphid number per natural enemy.

In each aphid treatment, Mann–Whitney U-tests with the Bonferroni adjustment for multiple tests were used for testing differences in the time spent by ants at each behaviour. The effect of the competition (single vs. multiple species treatment) and ant attendance on the per capita progeny production for each aphid species was analysed by two-way analysis of variance (ANOVA) after a $\log_{10}(x+1)$ transformation. The effect of the ant treatment on the natural enemy abundance was compared by χ^2-tests. All statistical analyses were performed using SPSS.

Results

Field experiments

Level of ant attendance. A total of 203 and 225 ants were recorded tending aphids in control trees in orchards 1 and 2, respectively. No ants were found in colonies of trees of the exclusion treatment in orchard 1, while seven ants were found in those trees in orchard 2. In orchard 1, the percentage of colonies tended by ants at each sampling date ranged between 30% and 50%, with a mean number of ants per colony of 4.95 (SE = 0.98). In orchard 2, between 50% and 80% of aphid colonies in control trees were tended by ants at each sampling date, with a mean number of ants per colony of 3.75 (SE = 0.50). In both orchards, the number of ants tending *D. plantaginea* colonies correlated significantly with colony size ($r_s = 0.357$, $P < 0.05$, $n = 41$ and $r_s = 0.523$, $P < 0.001$, $n = 59$ for orchards 1 and 2, respectively).

Effects of ant attendance on Dysaphis plantaginea *populations.* In orchard 1 there was a high natural mortality of fundatrices in both the presence and the absence of ants (Fig. 1a). However, in the absence of ants, extinction rate of aphid colonies was higher (Wilcoxon's signed-rank tests, $Z = -2.252$, $n = 8$, $P < 0.05$) and the aphid populations were smaller (ANOVAR, $F = 9.068$, d.f. = 1, 198, $P < 0.01$) (Fig. 1a).

Similarly, in orchard 2, the extinction of aphid colonies was significantly higher in the absence of ants (Wilcoxon's signed-rank tests, $Z = -2.521$, $n = 8$, $P < 0.05$). Although no effect of ant attendance was detected on the colony size (ANOVAR, $F = 1.807$, d.f. = 1, 47, $P = 0.185$; Fig. 1b), the total aphid counts in the presence of ants doubled that in the exclusion treatment (7335 vs. 3740).

Effects of ant attendance on Aphis *spp. and* Eriosoma lanigerum *populations.* No data were obtained from orchard 1 because neither *Aphis* spp. nor *E. lanigerum* were recorded in any of the marked shoots. However, these two aphid species did colonise experimental shoots in orchard 2 (Fig. 2). Despite the fact that ant attendance was recorded in at least 50% of *Aphis* colonies for all the sampling dates, no ant-effect was detected on the percentage of shoots in which this aphid occurred (Wilcoxon's signed-rank tests, $Z = -1.244$, $n = 9$, $P = 0.214$) nor on the colony size (ANOVAR, $F = 0.072$, d.f. = 1, 47, $P = 0.789$; Fig. 2a). On the contrary, *E. lanigerum* was present in a higher percentage on shoots in which ants were absent (Wilcoxon's signed-rank tests, $Z = -2.192$, $n = 10$, $P < 0.05$; Fig. 2b), although no effect was detected in the number of infested leaf axils (ANOVAR, $F = 1.676$, d.f. = 1, 47, $P = 0.202$).

Effects of ant attendance on the abundance of natural enemies. The colonies of *D. plantaginea* were exploited by a diverse guild of natural enemies (Table 1). The total number of predators recorded along the sampling period was 28% and 44% higher in the absence of ants in orchards 1 and 2, respectively ($\chi^2 = 5.21$, d.f. = 1, $P < 0.05$; $\chi^2 = 6.57$, d.f. = 1, $P < 0.05$ for orchards 1 and 2, respectively). On the contrary, the cumulative aphid number over the whole season was 56% and 49% higher in the control trees of orchards 1 and 2, respectively ($\chi^2 = 582.98$, d.f. = 1, $P < 0.001$; $\chi^2 = 537.85$, d.f. = 1, $P < 0.001$, for orchards 1 and 2, respectively). Consequently, the number of aphids per natural enemy was three-fold higher in the presence of ants in both orchards ($\chi^2 = 5.26$, d.f. = 1, $P < 0.05$; $\chi^2 = 32.29$, d.f. = 1, $P < 0.001$; for orchards 1 and 2, respectively; Table 1). As

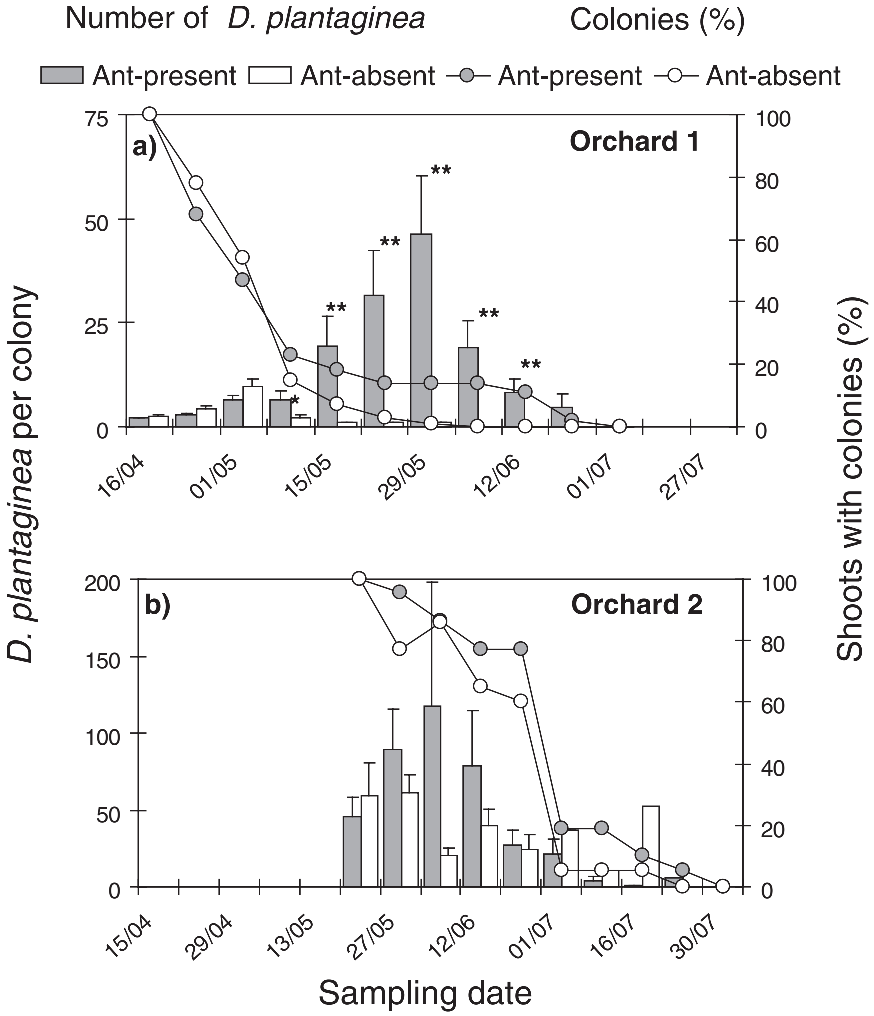

Fig. 1. Effect of the ant treatment on *Dysaphis plantaginea* in (a) orchard 1 and (b) orchard 2. Number of aphids per colony (mean + SE; bars) and percentage of shoots with colonies (lines) along the sampling period ($*P < 0.05$; $**P < 0.01$) are shown.

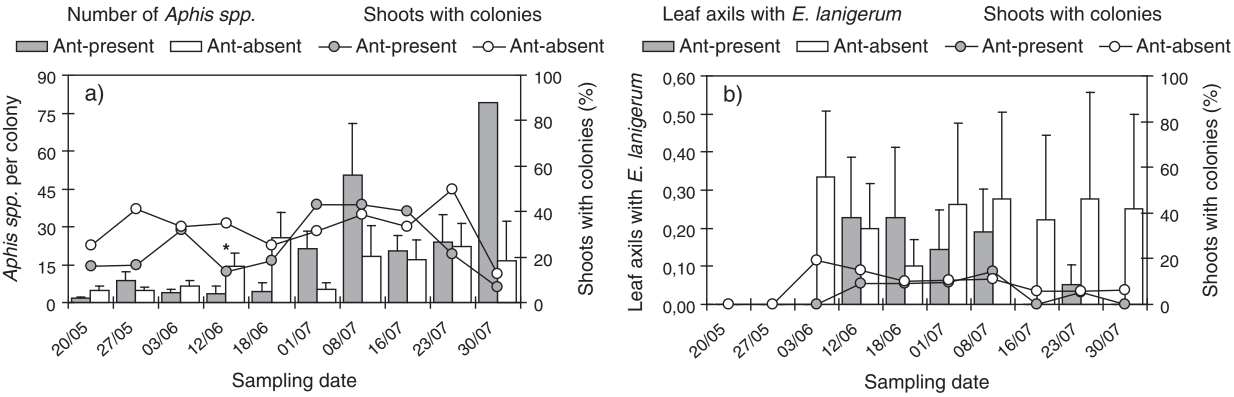

Fig. 2. Effect of the ant treatment on (a) *Aphis* spp. and (b) *Eriosoma lanigerum* in orchard 2. For *Aphis* spp., number of aphids per colony (mean + SE) and percentage of shoots with aphid colonies are shown; for *E. lanigerum*, number of leaf axils with aphids (mean + SE) and percentage of shoots with aphids are shown.

Table 1. Abundance of natural enemies on *Dysaphis plantaginea* colonies and ratio of aphids per natural enemy in the two experimental apple orchards according to the ant treatment.

		Orchard 1		Orchard 2	
Natural enemy	Life stage	Ant present	Ant absent	Ant present	Ant absent
Syrphidae	Eggs	89	160	4	12
	Larvae	19	18	21	14
	Pupae	1	2	–	–
Coccinellidae	Eggs	–	–	–	–
	Larvae	–	–	–	3
	Pupae	–	–	–	1
	Adults	1	–	1	2
Cecidomyiidae	Larvae	4	–	6	15
Anthocoridae	Nymphs + adults	–	–	3	3
Miridae	Nymphs + adults	11	1	1	–
Chrysopidae	Eggs	1	3	3	10
Forficulidae	Nymphs + adults	–	–	20	2
Cantharidae	Adults	4	1	–	4
Araneae		21	13	12	10
Mummies		4	–	2	29
Total antagonists		155	198^{*}	73	105^{*}
Cumulative aphid counts		2643	1155^{***}	7335	3740^{***}
Ratio aphids : natural enemy		17:1	$6:1^{*}$	100:1	$36:1^{***}$

$^{*}P < 0.05$.
$^{***}P < 0.001$.
χ^2 analysis by orchard.

a result, ant attendance was related to enhanced aphid numbers and to reduced enemy abundance, consequently increasing the number of aphids per natural enemy.

Manipulative experiment

Level of ant attendance and behaviour. The percentage of plants visited by ants at each control ranged between 50% and 90%. The total number of ants recorded along the experiment was 1411, with a mean number of ants per plant and control date of 9.22 (SE = 0.76).

Regarding ant behaviour, ants were found predominantly tending *Aphis* spp. (43–68% of time) or roaming (29–36%) (Table 2). When *Aphis* spp. and *D. plantaginea* were found together, ants spent more time tending *Aphis* spp. than *D. plantaginea* (Mann–Whitney, $U = 92.50$, $P < 0.01$ for the *D. plantaginea* treatment and $U = 10.50$, $P < 0.001$ for the coexistence treatment). Ants were never observed tending *E. lanigerum*. Other behaviours included cleaning, interacting with other ants, and collecting honeydew from leaves. Aphid predation by ants was not observed.

Effects of ant attendance and aphid competition on aphid populations. Exposure of aphids to ant attendance increased the abundance of *Aphis* spp. by 125% and reduced the abundance of *E. lanigerum* by 64% (Table 3; Fig. 3). No ant effect was detected on *D. plantaginea* populations. For *D. plantaginea* and *E. lanigerum*, there was no effect of the competition treatment, that is, per capita growth of aphids was the same for each species reared alone and in combination. However, there was a significant interaction between factors for *Aphis* spp. (Table 3; Fig. 3b): ants provided a benefit to *Aphis* when this aphid coexisted with the other two species (ANOVA, $F = 5.710$; d.f. = 1, 24; $P < 0.05$) but not in their absence (ANOVA, $F = 2.340$; d.f. = 1, 24; $P = 0.139$).

Effects of ant attendance on the abundance of natural enemies. For all plants, natural enemies were 60% more abundant when ants were absent than in their presence ($\chi^2 = 72.52$; $P < 0.001$; Table 4). This tendency was recorded for all the aphid treatments but no significant difference was found for plants infested with *E. lanigerum* only.

Discussion

Our results indicate that the dual role of ants as mutualists (of *D. plantaginea* and *Aphis* spp.) and antagonists (of *E. lanigerum*) can lead to changes in the structure of the aphid community on apple and can promote the coexistence of different apple species sharing the same host. This study also gives support to the observed pattern that ants can benefit tended aphids while at the same time reduce the abundance of untended herbivores (Styrsky & Eubanks, 2007) including other aphids (Smith *et al*., 2008).

In both the orchards and the manipulative experiment, ants were present and established different types of relationships with apple aphids. Ants were mutualistic of rosy apple aphid *D. plantaginea*, and the *Aphis* complex formed by green apple aphid, *A. pomi*, and spirea aphid, *A. spiraecola*, but antagonist of woolly apple aphid, *E. lanigerum*. As a result, populations of these aphids behaved differently in the presence and the absence of ants.

For *D. plantaginea* and *Aphis* spp. results differed between field and manipulative experiments. While ant attendance in the field increased *D. plantaginea* populations, no significant effect of ant attendance was detected in the manipulative experiment. On the contrary, although *Aphis* spp. colonised trees in one of the two experimental orchards and their colonies were tended, no ant effect on *Aphis* populations was detected in the field. However, ants improved the performance of *Aphis* spp. in the manipulative assay. Such differences between the two experimental periods, spring in the field and summer in the manipulative experiment, could be related to thermal tolerance, phenology, and life cycles.

With a temperature threshold for development of 4.5 °C (Graf *et al*., 1985), *D. plantaginea* is found on apple quite early in spring. After developing for some generations on apple, this species migrate to their secondary hosts typically in June (Bonnemaison, 1959; Miñarro *et al*., 2005). Thus the population decline observed for this species in the field can be attributed to the emigration to the secondary host. Similarly, the lower performance of this species in the manipulative conditions can be attributed to low tolerance to high temperatures (Graf *et al*., 1985) and to host alternation. In contrast, with a threshold of 5.9 °C (Graf *et al*., 1985), *A. pomi* populations develop later in the season and spend all its life cycle on apple. Attending to fecundity and nymph survival, Graf *et al*. (1985) concluded that *A. pomi* encountered more

Table 2. Interaction between ants and aphids represented as the percentage (mean ± SE) of time spent at each behaviour according to the aphid treatment. Note that *Aphis* spp. was present in all the treatments as a result of immigration of winged individuals.

	Aphid treatment			
Behaviour	*Aphis* spp.	*D. plantaginea*	*E. lanigerum*	Coexistence
Tending *Aphis* spp.	57.7 ± 9.3a	43.2 ± 9.3a	68.6 ± 9.3a	61.9 ± 6.3a
Tending *D. plantaginea*	Not present	13.5 ± 6.3b	Not present	3.9 ± 2.7d
Tending *E. lanigerum*	Not present	Not present	0.0 ± 0.0	0.0 ± 0.0
Predation	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Roaming	34.7 ± 9.0a	36.5 ± 7.7a	29.7 ± 9.0b	29.0 ± 5.7b
Others	7.6 ± 5.1b	6.8 ± 5.1b	1.7 ± 1.0c	5.2 ± 1.6c
Sample size	20	20	17	21

For each aphid treatment, data followed by the same letter are not different for $\alpha = 0.05$.

Table 3. Statistical results of the two-way ANOVA performed to test the effect of ant attendance and aphid competition on the aphid species.

	D. plantaginea		*Aphis* spp.		*E. lanigerum*	
Factors	*F*	*P*	*F*	*P*	*F*	*P*
Ants	0.89	0.35	8.23	**0.006**	14.93	<**0.001**
Competition	1.24	0.27	2.22	0.14	0.49	0.49
Ants × competition	2.16	0.15	5.77	**0.02**	0.01	0.92

For all tests, d.f. = 1, 48. Significant results ($P < 0.05$) are shown in boldface.

favourable conditions than *D. plantaginea* for development during warmer season. *Aphis spiraecola* is also benefited by high temperatures, since the optimal range of temperature was estimated between 20 and 30 °C (Wang & Tsai, 2000). Therefore, these *Aphis* species are frequently found on apple in summer as shown by the high number of winged immigrants arriving to apple plants during the manipulative experiment. These differences in thermal requirements could be an adaptive mechanism to reduce interspecific competition and to allow coexistence on the same resource (Speight *et al*., 2008).

When each species was grown in favourable conditions—*D. plantaginea* in spring in the field experiment and *Aphis* in summer in manipulative conditions—both were favoured by ant attendance. Ant disruption of natural control has been seen as the primary mechanism explaining the advantage for aphids from the ant–aphid mutualism (Banks, 1962; Bishop & Bristow, 2003; Nagy *et al*., 2007; Stewart-Jones *et al*., 2008). In our research, the presence of ants reduced the number of natural enemies and at the same time increased the abundance of aphids, consequently increasing the number of aphids per antagonist. Therefore, biological control of *D. plantaginea* and *Aphis* spp. could have been hampered by tending ants, as recently concluded by Nagy *et al*. (2007) and Stewart-Jones *et al*. (2008). However, results from the manipulative assay showed that ant–aphid mutualisms can be more complex. Despite a significant reduction of predators by 58% when *Aphis* was reared alone, ants did not provide a benefit to the aphid, since aphid abundance was similar in the presence and the absence of ants. However, in plants with the three aphid species together, ants reduced predators only by 21% but increased *Aphis* abundance by 256%. At the same time, populations of *E. lanigerum* decreased by 63% in the presence of ants and other aphid species. Therefore, ants only provided a benefit when the mutualist aphid was in competition, demonstrating that the benefit was not through reduction of predators (which occurred to a similar extent in each aphid treatment), but rather by reducing competition with other aphid species. This amelioration of the negative effects of competition is a novel mechanism explaining the benefit of ant attendance, as recently highlighted by Smith *et al*. (2008).

Contrarily to the other apple aphids, *E. lanigerum* was not tended by ants. A negative effect of ants on its populations was suggested in the field and confirmed in the manipulative experiment with an average reduction of 64% in the presence and absence of other aphids. This non-myrmecophilous aphid is protected against predators by the self-secreted wax (Moss *et al*., 2006). Actually, since the guild of natural enemies attacking *D. plantaginea* and *A. pomi* or *A. spiraecola* can be considered very similar and formed mainly by syrphids, coccinellids, cecidomyiids, chrysopids, and predatory heteropterans (this study, Miñarro *et al*., 2005; Brown & Matthews, 2007; Nagy *et al*., 2007; Fréchette *et al*., 2008; Stewart-Jones *et al*., 2008), the two most important enemies of *E. lanigerum* are the earwig *Forficula auricularia* L. and the specific parasitoid *Aphelinus mali* (Haldeman) (Mueller *et al*., 1988; Nicholas *et al*., 2005). We recorded earwigs but no *A. mali* parasitism in our experiments.

The exclusion method that used a sticky barrier on the trunks can have a negative impact on crawling predators such as earwigs (Piñol *et al*., 2009). Thus, the lower number of earwigs recorded in the ant-excluded trees in orchard 2 could partially explain the apparently higher abundance of *E. lanigerum* in those trees. However, no difference in earwig abundance which could explain the observed differences in aphid abundance in the presence and absence of ants was registered in the manipulative experiment. In addition, no aphid predation by ants was observed along the sampling period.

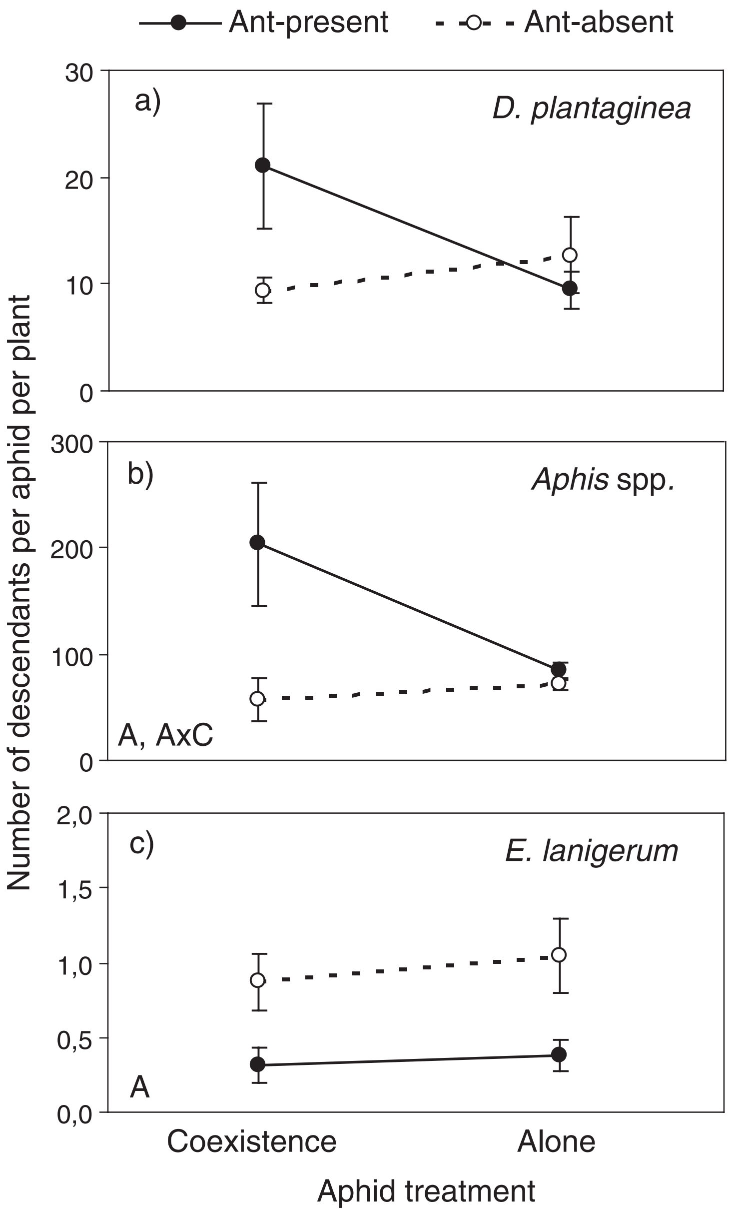

Fig. 3. Effects of ant attendance and competition for *Dysaphis plantaginea* (a), *Aphis* spp. (b), and *Eriosoma lanigerum* (c). The number of aphids per capita per plant (mean ± SE) are presented for the coexistence treatment (left) and for each aphid reared alone (right) in the presence (solid lines) and in the absence (dashed lines) of ants. A and A × C represent significant effects of the ant treatment and treatments interaction, respectively. Note that the vertical differences between lines represent the ant effect, while the slope of the lines represents the degree of the effect of the aphid treatment.

Thus, the mechanism by which *E. lanigerum* populations were reduced by ants is unclear, although direct aphid removal by ants should not be discarded since a reduction in aphid abundance was already detected just 2 days after ants visited the plants for the first time.

Ants are known to have an important effect on the abundance of the whole herbivore community living on plants, as well as in the balance of costs and benefits for the plant (Styrsky & Eubanks, 2007; Rosumek *et al*., 2009). When considering the net effect of ants on total aphid abundance (i.e. all the species pooled together), the per capita progeny production of aphids was two-fold higher in the presence than in the absence of ants [53.25 ± 12.59 (mean ± SE) vs. 25.34 ± 4.70; ANOVA, $F = 4.311$; d.f. = 1, 154; $P < 0.05$]. Thus, the overall balance between the contrasting effects of ants on tended and untended aphids on apple was a promotion of total aphid abundance. Similarly, in the review by Rosumek *et al.* (2009), the removal of ants caused a 66% reduction in honeydew producers on plants. In a community context of complex and contrasting interactions between ants, herbivores, and host plants, the net positive effect of ants on aphid abundance in our study could be related to an ant-mediated negative effect of aphids on apple tree fitness.

In conclusion, ants benefited the tended aphids while simultaneously reducing the abundance of the untended species. Through this contrasting effect, ants play a role in structuring the aphid community on apple. Ant-mediated reduction of the abundance of natural enemies as well as the also ant-mediated reduction of interspecific competition could be the mechanisms involved in the benefit of the ant–aphid

Table 4. Abundance of natural enemies for each aphid treatment in presence and absence of ants.

		Aphis spp.		*D. plantaginea*		*E. lanigerum*		Coexistence		Total	
Natural enemy	Life stage	Ant present	Ant absent	Ant present	Ant absent	Ant present	Ant absent	Ant present	Ant absent	Ant present	Ant absent
Syrphidae	Eggs	17	35	16	40	8	15	30	48	71	138***
	Larvae	22	32	9	7	6	9	14	19	51	67 NS
Coccinellidae	Eggs	7	25	–	45	15	20	14	7	36	97***
	Larvae	1	4	–	–	2	4	8	2	11	10 NS
	Adults	3	6	4	2	2	4	4	2	13	14 NS
Cecidomyiidae	Larvae	13	25	26	43	9	7	53	26	101	101 NS
Anthocoridae	Nymphs + adults	1	27	6	25	9	9	5	19	21	80***
Chrysopidae	Eggs	23	41	28	23	49	43	37	68	137	175*
	Larvae	1	3	3	2	3	1	1	–	8	6 NS
	Adults	–	3	1	1	–	–	–	1	1	5 NS
Forficulidae	Nymphs + adults	–	–	–	2	–	–	1	–	1	2 NS
Cantharidae	Adults	3	4	–	4	–	–	3	7	6	15*
Araneae		4	13	14	24	7	17	8	24	33	78***
Mummies		–	10	13	9	6	15	6	10	29	44 NS
Total		95	228***	120	227***	116	144 NS	184	233*	519	832***

$^{*}P < 0.05$.

$^{***}P < 0.001$.

NS, not significant.

χ^2 analysis for aphid treatment and total.

mutualism for the tended species. Although unclear, a direct predation on aphids by ants is supposed as the mechanism explaining the negative effect of ants on the untended species. Considering all the aphids together, ants had a net positive effect on aphid abundance, which in its turn could have a negative effect on the host plant, apple. However, the net effect of ants on the overall abundance of herbivores living on apple is still an open question.

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