

Context-dependence in an ant–aphid mutualism: direct effects of tending intensity on aphid performance

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Abstract. 1. In ant–hemipteran mutualisms, ants receive carbohydrates in the form of honeydew, while hemipterans receive protection from natural enemies. In the absence of natural enemies, however, the direct effects of tending are generally less well known. We hypothesised that with increasing tending intensity (ant to aphid ratio), aphid performance would increase initially, then decrease at high tending levels due to the metabolic cost of producing high quality honeydew.

2. We tested our hypothesis in a greenhouse experiment by manipulating Argentine ant (*Linepithema humile* Mayr) colony size while holding constant the initial size of aphid (*Chaitophorus populicola* Thomas) aggregations. The two parameters associated with survival, aphid survivorship to maturity and longevity, declined with increasing tending intensity, whereas per capita birth rate and time to first reproduction showed no relationship to attendance. The intrinsic rate of increase declined only at relatively high tending levels, suggesting a nonlinearity in the effect of tending intensity.

3. Tending intensity measured in the experiment was similar to that observed in free-living aggregations of *C. populicola*. Furthermore, the per capita recruitment rate of ants to free-living aphid aggregations was negatively density-dependent, indicating that small aggregations tend to experience the highest levels of tending intensity. This finding suggests that the aphid's intrinsic rate of increase may be positively density-dependent, mediated by the aphid's mutualistic interaction with the ant.

4. In the Argentine ant–*C. populicola* interaction, experimental manipulation of colony size revealed a direct cost of ant attendance that was conditional upon tending intensity. Experiments that manipulate only ant presence or absence may yield an incomplete understanding of the mutualistic interaction if underlying nonlinearities exist.

Key words. Ant : aphid ratio, ant attendance, conditionality, Hemiptera, life-history parameters, mutualist, myrmecophily, *Populus fremontii*.

Introduction

Mutualisms generally result in net benefits for the participants. It has become increasingly apparent, however, that the level of benefits conferred varies over time and space (Cushman & Whitham, 1989; Del-Claro & Oliveira, 2000; Billick & Tonkel, 2003; Gove & Rico-Gray, 2006). Gross costs and benefits are often context-dependent, resulting in net effects that may be density-dependent or variable under a range of biotic and abiotic factors that are extrinsic to the interaction (Breton & Addicott, 1992; Bronstein, 1994).

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Conditionality is an inherent feature of interactions between ants and honeydew-producing Hemiptera. Ant–hemipteran interactions typically involve the exchange of food for protection; ants obtain honeydew, a carbohydrate-rich resource, while hemipterans receive protection from natural enemies. Hemipterans can benefit from ant presence, however, even in the absence of natural enemies, indicating that benefits of tending may extend beyond protective services (Banks, 1962; Way, 1963; Breton & Addicott, 1992). Examples of beneficial effects include increased reproductive rate (Del-Claro & Oliveira, 2000; Flatt & Weisser, 2000), longevity (Flatt & Weisser, 2000), survivorship (Morales & Beal, 2006), development rate (Stadler & Dixon, 1999; Flatt & Weisser, 2000), or size (Bristow, 1984; Stadler & Dixon, 1999; Morales & Beal, 2006). The mechanisms underlying these

benefits include increased rates of phloem ingestion (leading to increased growth and reproduction; Way, 1963; Addicott, 1979; Morales, 2000a) and improved sanitation (Way, 1954; Wimp & Whitham, 2001). Such benefits may be dependent on the density of the hemipteran or ant partner, and may vary temporally (Billick & Tonkel, 2003).

Other studies have demonstrated a gross cost to ant tending. These costs are manifested in decreased rates of development (Stadler & Dixon, 1998), somatic growth (Yao *et al.*, 2000; Shibao *et al.*, 2009), reproduction (Yao *et al.*, 2000; Shibao *et al.*, 2009), and population growth (Johnson, 2008). Potential mechanisms underlying these costs include metabolic demands associated with the production of high quality honeydew or reduced nutrient assimilation at higher feeding and excretion rates (Yao & Akimoto, 2001; Katayama & Suzuki, 2002; Stadler & Dixon, 2005). Although not a direct cost of tending *per se*, tending ants may consume honeydew-producing hemipterans for their protein, when honeydew or alternative sources of carbohydrate are in excess (Way, 1954; Sudd, 1988; Sakata, 1995; Offenberg, 2001). In some facultatively tended Hemiptera, ant tending may not affect population growth at all (Herbert & Horn, 2008). Furthermore, tending may decrease dispersal through reduced movement or alate formation, although whether reduced dispersal is a benefit or cost can depend on the tradeoff between increased intraspecific competition and increased ant protection (Sudd, 1988; Oliver *et al.*, 2007).

Surprisingly, little is known about how the costs and benefits to hemipteran performance vary with respect to tending intensity (i.e. the ratio of ants to aphids in an aggregation). This key measure will potentially affect the rate at which individual aphids produce honeydew in response to tending by ants (Yao & Akimoto, 2001; Katayama & Suzuki, 2002), and can thus provide an indication of the physiological effect of ants on aphids. In general, the effects of one mutualist on another may vary linearly, saturate, or exhibit maxima at intermediate levels (Holland & DeAngelis, 2001; Holland *et al.*, 2002). The slope and shape of the relationship between the density of a mutualist and its effect on its partner can affect the stability of the mutualism, equilibrium densities (assuming stability), and whether or not the mutualism can become parasitic. A vast majority of studies examining these intrinsic effects, however, takes an all-or-nothing approach to the experimental manipulation of tending. That is, treatment levels for hemipterans comprise either fixed ant abundance, an unmanipulated ambient density of ants or complete isolation from ants (but see Way, 1954; Altfeld & Stiling, 2006). Other studies have used observational data to investigate the direct or indirect effects of at least two levels of tending intensity on parameters relating to hemipteran fitness (Cushman & Addicott, 1989; Flatt & Weisser, 2000).

In this study we experimentally manipulate tending intensity to quantify how this key aspect of ant-hemipteran mutualisms affects aphid performance. Our study system consists of the Argentine ant, *Linepithema humile* Mayr, the poplar leaf aphid, *Chaitophorus populicola* Thomas, and the Fremont Cottonwood, *Populus fremontii* S. Watson (Fig. 1). *Chaitophorus populicola* is autoecious and parthenogenetic, frequently occurs in dense aggregations on *Populus* throughout North



Fig. 1. Argentine ants tending *Chaitophorus populicola* on a *Populus fremontii* sapling. High levels of tending by the Argentine ant impose a direct cost to survivorship of *C. populicola*. Photograph by Ho Jung Yoo.

America (Solomon, 1986; Blackman & Eastop, 1994), and is commonly tended by the invasive Argentine ant in riparian habitats in southern California. Like other invasive ants, the Argentine ant often tends honeydew-producing insects (Way, 1963; Markin, 1970; Holway *et al.*, 2002; Ness & Bronstein, 2004) and has a high dietary requirement for carbohydrates (Grover *et al.*, 2007; Kay *et al.*, 2010). We hypothesised that tending at low levels of intensity would benefit aphid performance, potentially through improved sanitation service, which appears to be important for this prolific species (Wimp & Whitham, 2001), or through the stimulation of feeding rate. At higher levels of tending intensity, however, we expected that the high metabolic demand of tending might impose a cost on aphid performance, resulting in maximum performance at intermediate tending levels. To test our hypothesis, we manipulated the size of experimental ant colonies while holding initial aphid density constant. We then measured the reproductive and survivorship responses of an aphid cohort from birth to death. Concurrent with this experiment, we surveyed free-living aggregations of *C. populicola* throughout a growing season to characterise natural variation in aggregation size and tending intensity. The specific aim of this study was to clarify how different aspects of aphid fitness map onto a dynamic and poorly understood component of the mutualism. An overarching goal was to enhance our general understanding of the context-dependence of direct costs and benefits in mutualisms.

Materials and methods

Field survey

We surveyed Argentine ant-tended aggregations of *C. populicola* over one growing season (May to October 2008) to assess natural variation in tending intensity. These observations were conducted at Mission Trails Regional Park, San Diego County, CA, U.S.A. (32.8408°N, 117.0330°W). Every week throughout the survey we sampled approximately 30 aphid aggregations and recorded their size, the number of tending

ants, and the presence of natural enemies. All aggregations were located on terminal flushes of mature *P. fremontii*, and sampling was limited to three or fewer terminal flushes per tree per visit, with a maximum of one flush per primary branch (i.e. a structural limb emanating from the trunk). When aggregations went extinct, we included new aggregations to keep weekly counts at ca. 30 aggregations until late in the season, when regional population declines made it difficult to find any additional aphids. The survey data were used to calculate the mean ant-to-aphid ratio for each aggregation across every survey date within each month. For direct comparison to the *Greenhouse experiment* (see next section), mean aphid and ant abundances and their ratio were also calculated for each aggregation across sample dates falling within the same time period (23 July 2008 to 2 September 2008) as that used in the greenhouse experiment.

Greenhouse experiment

To quantify how the performance of *C. populicola* aggregations changes as a function of ant tending intensity, we conducted a manipulative experiment in the greenhouse in which we reared *C. populicola* on *P. fremontii* saplings grown in pots containing Argentine ant colonies of different sizes (from 0 to 1000 workers). Each sapling ($n = 48$) was grown in a 3.8-litre pot to which 180 ml of water was added daily for the duration of the experiment. At the start of the experiment saplings were 0.5 ± 0.1 m (mean \pm SD) in height and supported 47 ± 13 leaves.

Argentine ant colonies used in this experiment came from eight different riparian sites located throughout coastal San Diego County; collecting sites were separated by at least 1 km. We sorted material collected from every site into a single experimental colony of each of the following sizes: 10 workers + 5 brood, 50 workers + 25 brood, 100 workers + 50 brood, 500 workers + 100 brood, or 1000 workers + 100 brood. Each experimental colony had three queens. Each of the five colony size categories was thus replicated eight times; a set of eight replicates that had no ants was also included. Ants were added to potted *P. fremontii* saplings just before the start of the experiment; ants readily nested in soil contained in each pot. Potted saplings were placed in circular plastic containers lined with Fluon[®] to keep ants from escaping. Each container was further isolated over a shallow, water-filled moat. For the duration of the experiment, ants were provided with a protein source (one to two crickets per pot) twice weekly.

To inoculate experimental cottonwood saplings with *C. populicola*, we first initiated a source colony originating from >300 aphids collected on mature *P. fremontii* at Mission Trails Regional Park in June 2008. We reared the aphids on *P. fremontii* saplings growing outdoors in 19 litre pots until the start of the experiment. Before these aphids were discovered by free-living Argentine ant colonies, they reproduced rapidly and quickly fouled potted *P. fremontii* saplings with honeydew. On 23 July 2008 ('Day 0' of the experiment), 9–10 apterous adult *C. populicola* per replicate were transferred onto leaves on the top third of each cottonwood sapling; aphids were

initially enclosed in 2-cm diameter clip cages. After 2 days, clip cages and all adult aphids were removed. Nymphs were either removed or transferred among plants, as needed, so that each sapling contained exactly 20 1- to 2-day-old nymphs (F1 generation).

Within the greenhouse, we placed the 48 replicates along two benches, distributed spatially in complete randomised blocks. Every 3–4 days we rotated the position of blocks within and between greenhouse benches to minimise differences in microclimatic conditions among replicates. Greenhouse temperature was maintained at 22.5–34.6 °C (mean = 27.1 °C). Relative humidity ranged from 57.1 to 90.0% (mean = 76.9%).

Plants were checked once daily, between 09.00 and 12.00 hours, and the following data were recorded for each replicate: the number of ants present on each plant within a 10-s observation, total number of surviving F1 aphids, and number of adult aphids (due to maturation of F1 nymphs). Offspring produced by F1 aphids were also counted and removed daily. After daily reproduction rate peaked (in mid-August), plants were checked every other day. The experiment was terminated on 2 September.

Statistical analysis

To address the question of how aphid performance varies with tending intensity in the absence of predation and parasitism, we first defined the main explanatory variable, *tending intensity*, as the ratio of the number of ants foraging on a plant relative to the number of aphids present on the plant. For each replicate, daily measures of tending intensity were averaged over time. For this ratio, we included all ants that were on plants but not necessarily within the immediate vicinity of aphids, because (i) we assumed that Argentine ant workers were foraging on the plant primarily in search of honeydew (Grover *et al.*, 2008), and (ii) we could not easily distinguish, for workers that were in the immediate vicinity of aphids, how many individuals had recently palpated an aphid.

Aphid performance was quantified using seven life history measures: nymphal survivorship to maturity, time to first reproduction, mean daily per capita reproduction, longevity, net reproductive rate (R_0), generation time (G), and intrinsic rate of population increase (r). Survivorship to maturity was estimated as the maximum number of adult aphids counted on each plant during the experiment. Although this measure would not necessarily account for additional nymphal maturation offset by same-day adult mortality that may have masked a greater survivorship to adult, we assumed that such turnover was minimal and equivalent across replicates, and that the reproductive value of such short-lived adults is of minimal importance to overall aphid performance measures. Time to first reproduction was estimated from the number of days between adult introduction into clip cages (Day 0) and the first day at least one neonate was found on the plant. Mean daily per capita reproduction was calculated as the average number of neonates produced per adult female per day until extinction of F1 adults. Longevity was calculated as the mean number of days since Day 0 to

death of each F1 individual; death was either confirmed by discovery of cadavers or presumed from drops in daily counts. For the latter portion of the experiment, when counts were performed every 2 days, both neonate production rate and mortality were assumed to be constant over the previous 2-day interval between counts. For each replicate, R_0 was calculated via life table analysis as the sum of daily $l_x \times b_x$ products for the duration of the experiment, where l_x equals survivorship of F1 individuals to each age class (x days) and b_x equals per capita reproduction per F1 adult (Gotelli, 1998). The intrinsic rate of increase was approximated as $r \approx \ln(R_0)/G$, where

$$G = \frac{\sum_{i=1}^k (l_x \times b_x \times x)}{\sum_{i=1}^k (l_x \times b_x)}$$

for all $k = 41$ days of the experiment. By day 41, a mean of one adult aphid remained in replicates, and these survivors produced less than one neonate per day. Aphid populations that had not gone extinct in replicates before the last count on day 41 were assumed to have died by the next scheduled count, 2 days after the last actual count.

The first six measures of aphid performance were treated as response variables in separate generalised linear models (Proc GENMOD, SAS Institute, 2008), each including three explanatory variables: mean tending intensity (ant : aphid ratio), ant collection site, and their interaction. For survivorship to adult, tending intensity was averaged for each replicate up to the date that 100% of surviving nymphs reached maturity. For time to first reproduction, tending intensity was averaged up to the date that the first neonate was produced. For all other aphid performance measures, tending intensity was averaged over all dates of the experiment until the F1 generation went extinct. A logistic regression with binomial error distribution and logit link function was used in the analysis of proportional data, and Poisson regressions with a Poisson error distribution and log link function were used for count data. In all Poisson regressions, overdispersed and underdispersed data were corrected by scaling with Pearson's χ^2 (Allison, 1999). The effect of tending intensity on intrinsic rate of increase was first assessed using a simple regression of r against tending intensity, followed by a second-order polynomial regression. The Akaike Information Criterion (AIC) was then used to select the better supported of these two models.

Results

Field survey

Of the 350 separate weekly counts of *C. populicola* aggregations, only 14 (4%) were untended by ants. Untended aggregations had a mean (\pm SE) abundance of 6.86 ± 2.24 aphids, whereas tended aggregations contained 52.51 ± 3.53 aphids, a higher abundance in comparison ($t = 10.92$, d.f. = 127, $P < 0.0001$). Although mean numbers of ants and aphids in individual aggregations increased 4- to 2.5-fold, respectively, from May to October, the mean ant-to-aphid ratio remained within

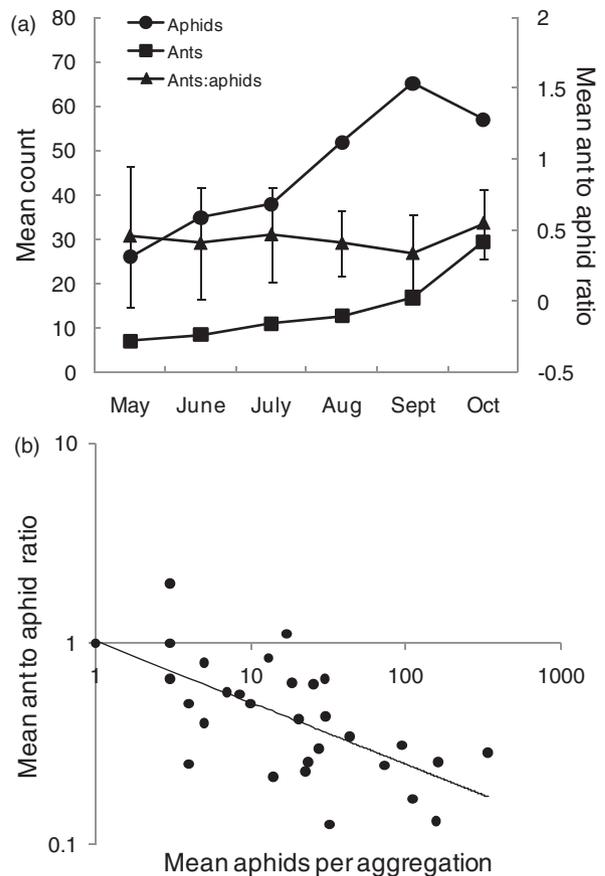


Fig. 2. (a) Mean monthly ant and aphid counts, and their ratio, per aggregation in samples taken weekly at Mission Trails Regional Park. Bars on ratio data represent ± 1 SD. Standard deviations of the count data are not shown, to preserve clarity of the plot. (b) Mean relative recruitment of ants as a function of mean aphid abundance in free-living aggregations sampled weekly during the period 23 July to 2 September 2008 (least squares fit through logged data: $y = -0.31x + 0.01$).

relatively narrow bounds (range: 0.34–0.55; mean \pm SE over all dates = 0.45 ± 0.03) (Fig. 2a). The logged ratio of ants to aphids declined significantly with increasing log of aphid count per colony (Fig. 2b, $F = 19.95$, d.f. = 1, 28, $P = 0.0001$).

Natural enemy abundances in aggregations were low. Ten out of the 88 aphid colonies showed evidence of parasitic activity (i.e. ≥ 1 aphid mummy present) on at least one survey date. We observed mummies during all survey months, but only 18 out of 17 757 recorded aphids across survey dates were mummified. Mummies appeared in aphid aggregations with tending ratios that ranged from 0 to 1 ant per aphid; ant numbers present in these aggregations ranged from 0 to 112. In 350 weekly observations of aphid aggregations, we observed potential predators within or in the immediate vicinity of aggregations on 13 occasions. Predator observations included counts of the following taxa: four coccinellid larvae, six syrphid larvae, four syrphid pupae, and one spider. In all cases, aphids in these aggregations were being tended by ants, but in no case did we see ants interacting with the predators.

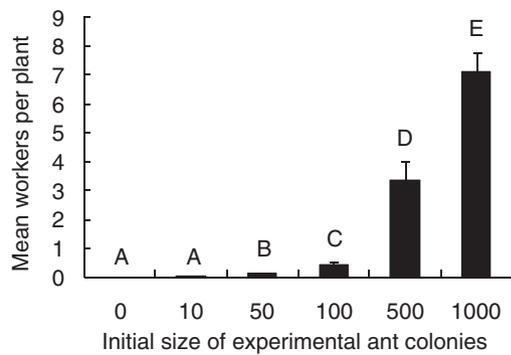


Fig. 3. Effect of initial colony size on the mean (\pm SE) number of workers foraging on plants in the greenhouse experiment. Different letters above bars indicate significantly different treatment effects based on multiple comparison Mann–Whitney tests (with Bonferroni correction) following a Kruskal–Wallis test.

Greenhouse experiment

The mean daily number of ants found on plants differed across treatments (Kruskal–Wallis: $\chi^2 = 43.74$, d.f. = 5, $P < 0.0001$), and all treatments with the exception of the experimental groups with 0 and 10 workers were significantly different from one another (Fig. 3; Mann–Whitney tests with Bonferroni correction). In the greenhouse experiment, as in the field, tending intensity was most commonly between zero and one ant per aphid, with occasional instances of one to two ants per aphid. The mean ant-to-aphid ratio in the four largest treatments was 0.36 ± 0.08 (cf. Fig. 2a). Means were calculated over time from the four largest treatments because only experimental groups with ≥ 50 workers resulted in ant tending levels significantly greater than zero (Fig. 3).

Four of the six variables related to aphid performance exhibited significant declines with increasing tending intensity. The two parameters associated with survival, survivorship to maturity and longevity, both declined with increasing tending intensity (Table 1, Fig. 4a, b), whereas the two parameters associated with reproduction did not (Table 1). Both R_0 and G decreased significantly with increasing mean tending intensity (Table 1, Fig. 4c,d). Generation time also varied among ant collection sites (Table 1), but not with respect to the interaction between collection site and tending intensity. Neither ant collection site nor its interaction with tending intensity had significant effects on aphid survivorship to maturity, time to first reproduction, longevity, or R_0 (Table 1). The data points with mean ant to aphid ratio > 1 did not exert statistically significant influence (i.e. Cook's $D_i > F_{0.50(1),4,44}$) on any of the four relationships depicted in Fig. 4a–d.

A linear model fit to the data indicated that r declined significantly with increasing mean tending intensity (slope = -0.033 , $F = 7.04$, d.f. = 1, 46, $P = 0.0109$, $R^2 = 0.13$). A slightly improved fit to the data was obtained with a second-order polynomial regression model (Fig. 5; $F = 6.83$, d.f. = 2, 45, $P = 0.0026$, $R^2 = 0.23$) whose AIC value, -184.4 , is smaller than -180.5 for the linear model. The nonlinear relationship indicates an increasingly negative relationship between r and tending intensity with higher levels of tending.

Discussion

Mutualisms are dynamic, context-dependent interactions between organisms that can incur both gross benefits as well as gross costs to the interacting partners. These costs and benefits may increase or decrease in a linear or nonlinear manner with the density of either partner, with subsequent net effects on the

Table 1. Effects of tending intensity on parameters relating to aphid performance.

Response variable	Error distribution	Explanatory variables	Estimate	SE	χ^2	d.f.	P
Survivorship to adult	Binomial	Ant : aphid ratio [†]	-1.3445	1.1586	4.82	1, 32	0.0281
		Ant site	—	—	5.48	7, 32	0.6021
		Ratio \times ant site	—	—	1.44	7, 32	0.9843
Mean per capita reproduction	Poisson	Ant : aphid ratio	-0.1820	0.1660	1.82	1, 32	0.1775
		Ant site	—	—	4.15	7, 32	0.7618
		Ratio \times ant site	—	—	23.22	7, 32	0.0016
Time to first reproduction	Poisson	Ant : aphid ratio [‡]	0.0794	0.1567	0.69	1, 32	0.4076
		Ant site	—	—	1.88	7, 32	0.9661
		Ratio \times ant site	—	—	10.96	7, 32	0.1403
Longevity	Poisson	Ant : aphid ratio	-0.5240	0.3476	9.48	1, 32	0.0021
		Ant site	—	—	5.29	7, 32	0.6247
		Ratio \times ant site	—	—	1.32	7, 32	0.9879
Net reproductive rate, R_0	Poisson	Ant : aphid ratio	-1.3400	0.7214	9.95	1, 32	0.0016
		Ant site	—	—	7.24	7, 32	0.4041
		Ratio \times ant site	—	—	1.70	7, 32	0.9744
Generation time, G	Poisson	Ant : aphid ratio	-0.1605	0.1161	12.27	1, 32	0.0005
		Ant site	—	—	15.14	7, 32	0.0343
		Ratio \times ant site	—	—	12.77	7, 32	0.0778

[†]Mean ant : aphid ratio was calculated for the period from Day 0 to the date of 100% maturity in each replicate.

[‡]Mean ant : aphid ratio was calculated for the period from Day 0 to the date of first reproduction in each replicate.

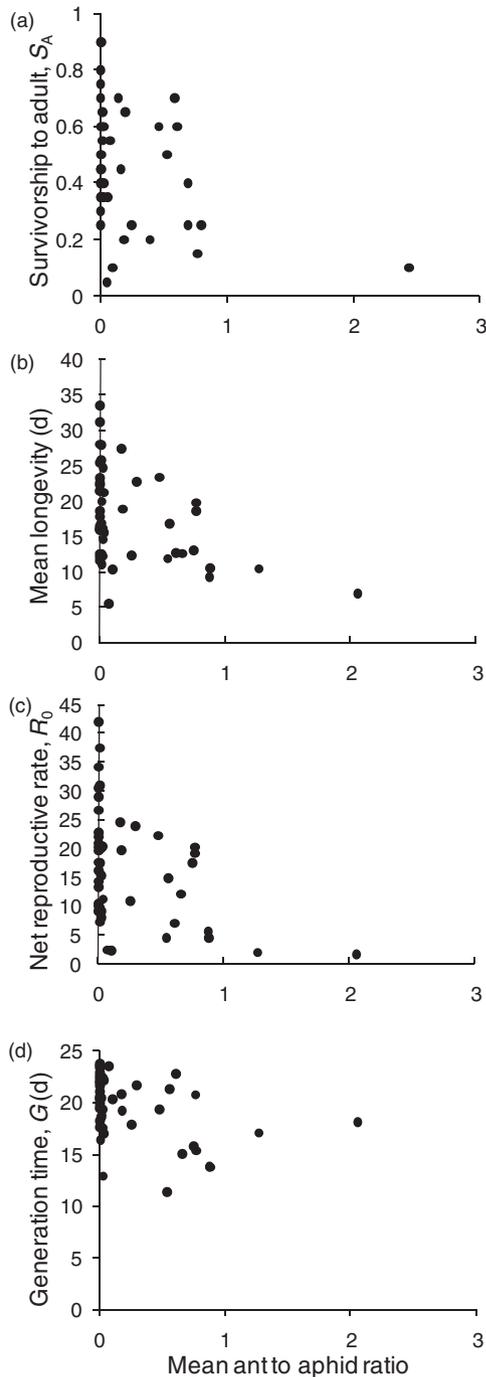


Fig. 4. Results of the greenhouse experiment. Effects of tending intensity on (a) survivorship of F1 nymphs to adult, (b) mean longevity of F1 aphids, (c) aphid net reproductive rate, and (d) aphid generation time. Multiple regression statistics are given in Table 1.

interaction (Bronstein, 1994; Holland & DeAngelis, 2001). In the association between the Argentine ant and *C. populicola*, tending in the absence of natural enemies incurred a direct cost to aphid population growth. By manipulating the number

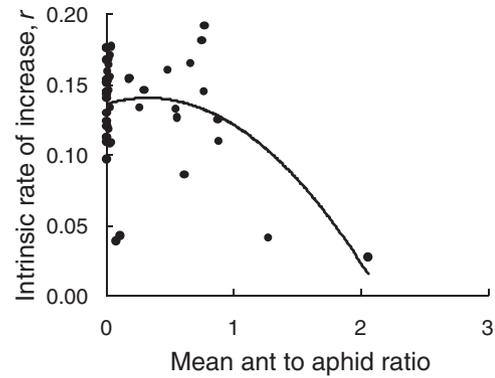


Fig. 5. Effect of tending intensity on aphid intrinsic rate of increase. The solid line is a second-order polynomial regression fit by the least squares method: $y = 0.136 + 0.027x - 0.042x^2$.

of tending ants per aphid, we showed support for our hypothesis that the cost of ant attendance is greater for aphids at higher levels of tending intensity than at lower levels. On the other hand, the aphid's intrinsic rate of increase appeared to show little response to tending intensity at low tending ratios (Fig. 5). In contrast to our expectations, tending did not appear to benefit the aphid at low levels of intensity, suggesting that the aphid benefits from its interaction with the ant primarily through indirect effects, i.e. protection from natural enemies. The experimental design thus revealed not only a gross cost of tending, but also nonlinearity in the effect of tending intensity on aphid performance.

The range of tending intensities generated in the experiment overlapped strikingly with tending levels experienced by aphids in the field (Figs 2b and 4a, b). The majority of aggregations in the field had ratios in the range of 0 to 1, where tending intensity has a negligible direct effect on intrinsic rate of increase. Thus, tending in the field may typically impose little direct cost to aphids. At the higher ratios that occur occasionally in small aggregations (Fig. 2b), however, aphids may suffer costs in terms of longevity.

The negative effect of tending intensity on net reproductive rate, the number of F2 females produced per F1 female, was primarily due to a reduction in longevity of F1 individuals and survivorship to adult (Table 1, Fig. 4a, b). Tending had no effect on per capita birth rate or developmental time to maturity (Table 1). We do not have a mechanistic explanation for why *C. populicola* suffered a survivorship rather than reproductive cost to tending; however, aphids are clonal organisms selected for rapid and prolific reproduction rather than individual survival. Further, tending by ants can take a physiological toll on honeydew-producing insects. Studies have shown that honeydew production rate and composition can be affected by stimulation from tending ants (Takeda *et al.*, 1982; Yao & Akimoto, 2001, 2002; Katayama & Suzuki, 2002; Stadler & Dixon, 2005). Increasing honeydew production may reduce an aphid's assimilation efficiency or increase stress as a result of the high physiological demands of ingestion and excretion during attendance (Stadler & Dixon, 1998; Yao & Akimoto, 2001). The proportion of oligosaccharides (e.g.

melezitose) in honeydew preferred by ants (Kiss, 1981; Volk *et al.*, 1999; Woodring *et al.*, 2004) can be high in ant-tended *Chaitophorus* spp. (Fischer & Shingleton, 2001). Such sugars are likely costly to produce, as production of melezitose is reported to decrease in *Chaitophorus* when not tended (Fischer & Shingleton, 2001). A negative direct effect of tending has been demonstrated in a few other aphid species, although the costs were incurred in reproduction or developmental parameters rather than survivorship (Stadler & Dixon, 1998; Yao *et al.*, 2000).

Reduced survivorship in our study is unlikely to result from either predation on the aphid by the Argentine ant or disturbance of aphids by ants, for several reasons. First, predation on *C. populicola* by the Argentine ant and aphid drop from the plant were rarely observed in the field or greenhouse. Second, killed crickets provided a source of protein *ad libitum* throughout the experiment, and no alternative sources of carbohydrates other than from aphid honeydew were made available. The Argentine ant has a high demand for carbohydrates (Grover *et al.*, 2007; Kay *et al.*, 2010). Without alternative sources of carbohydrates in the experimental arenas (Offenberg, 2001), the Argentine ant is more likely to have utilised aphids as carbohydrate rather than protein sources. Third, dramatic declines in aphid numbers (i.e. ≥ 5 individuals dying in 1 day), which might be expected in pots where colonies switched from tending to predation, occurred in only three replicates, one each from the 0, 100, and 500 initial worker treatments. Finally, previous experimental work with various aphid species has shown that the strongly myrmecophilous *C. populicola* does not fall off plants when exposed to alarm pheromones typically released by a variety of aphid species when disturbed (Nault *et al.*, 1976). Thus, neither ant activity nor manual removal of F2 nymphs is likely to have caused disturbance to aphid colonies leading to significant aphid drop from plants, consistent with our observations.

The direct effect of Argentine ant on *C. populicola* is context-dependent within the density ranges utilised in the experiment, and potentially parasitic in the absence of natural enemies. In nature, the presence of a natural enemy community likely results in net benefits to aggregations of *C. populicola* that are protected by tending ants (Wimp & Whitham, 2001). For example, the Argentine ant responds to alarm pheromones emitted by *C. populicola* (Mondor & Addicott, 2007), suggesting that myrmecophily is an important mechanism for reduction of mortality risk from natural enemies. Interestingly, however, pressure from natural enemies such as larval coccinellids, syrphids, and parasitoids did not appear to be substantial at our field site. If the Argentine ant provides effective protective services, then the most important natural enemies in this system are unlikely to be the taxa and developmental stages observed within the surveyed aggregations. Thus, the greatest potential benefit conferred by ants on *C. populicola* may be protection from highly mobile natural enemies such as adult coccinellids, anthocorids, and larval chrysopids that may have lower residence times in aggregations or that may be more susceptible to harassment from ants (Bartlett, 1961; Banks, 1962; Herbert & Horn, 2008). Another potential benefit is sanitation service, which prevents the drowning of

aphids in honeydew when aphid densities are high (Way, 1954; Wimp & Whitham, 2001). Finally, untended aggregations were rare, and, on average, smaller than tended aggregations. It is unclear without further experimentation whether aphids cannot survive in the absence of ant protection (i.e. the aphid is an obligate myrmecophile) or if the Argentine ant is simply very efficient at finding and recruiting to aggregations of *C. populicola*.

Interestingly, larger aggregations of aphids and membracids typically recruit a diminishing per capita number of tending ants (Fig. 2b; see also Addicott, 1979; Cushman & Whitham, 1991; Breton & Addicott, 1992; Morales, 2000b; Bluthgen *et al.*, 2006; Gove & Rico-Gray, 2006; Harmon & Andow, 2007). This negative density-dependence in per capita ant recruitment means that small aggregations of *C. populicola* experience the greatest levels of tending intensity (Fig. 2b), which can result in lower intrinsic rate of increase (Fig. 5) relative to larger aggregations with lower per capita tending. Whether this positive density-dependence in r leads to positive density-dependence in realised aphid population growth rate will largely depend on how protective service by ants varies with tending intensity. If the level of protection tends to decline with decreasing tending intensity, then mortality risk in aphids from natural enemies could be positively density-dependent. Subsequently, the net effect of tending on aphid population growth rate may be density-independent or negatively density-dependent, depending on the relative magnitudes of direct (physiological) and indirect (protective) effects of tending on the aphid. Only by understanding the full range of gross benefits and costs can one begin to predict net effects of one partner on the other in these widespread mutualisms.

Acknowledgements

We thank Andrea Drager and John Ludka for help in setting up the experiment, and the rangers at Mission Trails Regional Park for permission to work at the site. Katie LeVan offered helpful comments on the manuscript. The work was supported by a USDA grant (NRI-CGP2006-35302-17255) to D.A.H.

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Accepted 13 April 2011

First published online 1 June 2011