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

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Running head: Effects of tending on aphid performance

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 hypothesized 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*) colony size while holding constant the initial size of aphid (*Chaitophorus populicola*) 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 which 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 attendance, ant:aphid ratio, conditionality, Hemiptera, life history parameters, mutualist, myrmecophily, *Populus fremontii*

1 **Introduction**

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

8 Conditionality is an inherent feature of interactions between ants and honeydew-
9 producing Hemiptera. Ant-hemipteran interactions typically involve the exchange of food for
10 protection; ants obtain honeydew, a carbohydrate-rich resource, while hemipterans receive
11 protection from natural enemies. Hemipterans can benefit from ant presence, however, even in
12 the absence of natural enemies, indicating that benefits of tending may extend beyond protective
13 services (Banks 1962; Way 1963; Breton & Addicott 1992). Examples of beneficial effects
14 include increased reproductive rate (Del-Claro & Oliveira 2000; Flatt & Weisser 2000),
15 longevity (Flatt & Weisser 2000), survivorship (Morales & Beal 2006), development rate
16 (Stadler & Dixon 1999; Flatt & Weisser 2000), or size (Bristow 1984; Stadler & Dixon 1999;
17 Morales & Beal 2006). The mechanisms underlying these benefits include increased rates of
18 phloem ingestion (leading to increased growth and reproduction; Way 1963; Addicott 1979;
19 Morales 2000a) and improved sanitation (Way 1954; Wimp & Whitham 2001). Such benefits
20 may be dependent on the density of the hemipteran or ant partner, and may vary temporally
21 (Billick & Tonkel 2003).

22 Other studies have demonstrated a gross cost to ant tending. These costs are manifested
23 in decreased rates of development (Stadler & Dixon 1998), somatic growth (Yao, Shibao &

Akimoto 2000; Shibao *et al.* 2009), reproduction (Yao, Shibao & Akimoto 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, DeAngelis & Bronstein 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

1 hemipterans comprise either fixed ant abundance, an unmanipulated ambient density of ants or
2 complete isolation from ants (but see Way 1954, Altfeld & Stiling 2006). Other studies have
3 used observational data to investigate the direct or indirect effects of at least two levels of
4 tending intensity on parameters relating to hemipteran fitness (Cushman & Addicott 1989; Flatt
5 & Weisser 2000).

6 In this study we experimentally manipulate tending intensity to quantify how this key
7 aspect of ant-hemipteran mutualisms affects aphid performance. Our study system consists of the
8 Argentine ant, *Linepithema humile* (Mayr), the poplar leaf aphid, *Chaitophorus populicola*
9 Thomas, and the Fremont Cottonwood, *Populus fremontii* S. Watson (Plate I). *Chaitophorus*
10 *populicola* is autoecious and parthenogenetic, frequently occurs in dense aggregations on
11 *Populus* throughout North America (Solomon 1986; Blackman & Eastop 1994), and is
12 commonly tended by the invasive Argentine ant in riparian habitats in southern California. Like
13 other invasive ants, the Argentine ant often tends honeydew-producing insects (Way 1963;
14 Markin 1970; Holway *et al.* 2002, Ness & Bronstein 2004) and has a high dietary requirement
15 for carbohydrates (Grover *et al.* 2007, Kay *et al.* 2010). We hypothesized that tending at low
16 levels of intensity would benefit aphid performance, potentially through improved sanitation
17 service, which appears to be important for this prolific species (Wimp & Whitham 2001), or
18 through the stimulation of feeding rate. At higher levels of tending intensity, however, we
19 expected that the high metabolic demand of tending might impose a cost on aphid performance,
20 resulting in maximum performance at intermediate tending levels. To test our hypothesis, we
21 manipulated the size of experimental ant colonies while holding initial aphid density constant.
22 We then measured the reproductive and survivorship responses of an aphid cohort from birth to
23 death. Concurrent with this experiment, we surveyed free-living aggregations of *C. populicola*

1 throughout a growing season to characterize natural variation in aggregation size and tending
2 intensity. The specific aim of this study was to clarify how different aspects of aphid fitness map
3 onto a dynamic and poorly understood component of the mutualism. An overarching goal was to
4 enhance our general understanding of the context-dependence of direct costs and benefits in
5 mutualisms.

6

Materials and methods

Field survey

We surveyed Argentine ant-tended aggregations of *C. populicola* over one growing season (May – October 2008) to assess natural variation in tending intensity. These observations were conducted at Mission Trails Regional Park, San Diego County, CA, USA (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 – 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 L pot to

1 which 180 mL of water was added daily for the duration of the experiment. At the start of the
2 experiment saplings were 0.5 ± 0.1 m (mean $\pm$ SD) in height and supported 47 ± 13 leaves.

3 Argentine ant colonies used in this experiment came from eight different riparian sites
4 located throughout coastal San Diego County; collecting sites were separated by at least 1 km.
5 We sorted material collected from every site into a single experimental colony of each of the
6 following sizes: 10 workers + 5 brood, 50 workers + 25 brood, 100 workers + 50 brood, 500
7 workers + 100 brood, or 1000 workers + 100 brood. Each experimental colony had 3 queens.
8 Each of the five colony size categories was thus replicated eight times; a set of eight replicates
9 that had no ants was also included. Ants were added to potted *P. fremontii* saplings just before
10 the start of the experiment; ants readily nested in soil contained in each pot. Potted saplings were
11 placed in circular plastic containers lined with Fluon® to keep ants from escaping. Each
12 container was further isolated over a shallow, water filled moat. For the duration of the
13 experiment, ants were provided with a protein source (1-2 crickets per pot) twice weekly.

14 To inoculate experimental cottonwood saplings with *C. populicola*, we first initiated a
15 source colony originating from > 300 aphids collected on mature *P. fremontii* at Mission Trails
16 Regional Park in June 2008. We reared the aphids on *P. fremontii* saplings growing outdoors in
17 19 L pots until the start of the experiment. Before these aphids were discovered by free-living
18 Argentine ant colonies, they reproduced rapidly and quickly fouled potted *P. fremontii* saplings
19 with honeydew. On 23 Jul 2008 (“Day 0” of the experiment), 9-10 apterous adult *C. populicola*
20 per replicate were transferred onto leaves on the top third of each cottonwood sapling; aphids
21 were initially enclosed in 2-cm diameter clip cages. After two days, clip cages and all adult
22 aphids were removed. Nymphs were either removed or transferred among plants, as needed, so
23 that each sapling contained exactly 20 one- to two-day old nymphs (F1 generation).

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

6 Plants were checked once daily, between 0900 h and 1200 h, and the following data were
7 recorded for each replicate: the number of ants present on each plant within a 10-second
8 observation, total number of surviving F1 aphids, and number of adult aphids (due to maturation
9 of F1 nymphs). Offspring produced by F1 aphids were also counted and removed daily. After
10 daily reproduction rate peaked (in mid-August), plants were checked every other day. The
11 experiment was terminated on 2 September.

13 *Statistical analysis*

14 To address the question of how aphid performance varies with tending intensity in the absence of
15 predation and parasitism, we first defined the main explanatory variable, *tending intensity*, as the
16 ratio of the number of ants foraging on a plant relative to the number of aphids present on the
17 plant. For each replicate, daily measures of tending intensity were averaged over time. For this
18 ratio, we included all ants that were on plants but not necessarily within the immediate vicinity
19 of aphids, because 1) we assumed that Argentine ant workers were foraging on the plant
20 primarily in search of honeydew (Grover *et al.* 2008), and 2) we could not easily distinguish, for
21 workers that were in the immediate vicinity of aphids, how many individuals had recently
22 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 two days, both neonate production rate and mortality were assumed to be constant over the previous two-day interval between counts. For each replicate, R_0 was calculated via life table analysis as the sum of daily $l_x * b_x$ 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

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

for all $k = 41$ days of the experiment. By day 41, a mean of 1 adult aphid remained in replicates, and these survivors produced less than 1 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, two days after the last actual count.

The first six measures of aphid performance were treated as response variables in separate generalized linear models (Proc GENMOD, SAS Institute, Cary, NC, USA), 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 chi-square (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-fold to 2.5-fold, respectively, from May to October, the mean ant-to-aphid ratio remained within relatively narrow bounds (range: 0.34 - 0.55; mean $\pm$ SE over all dates = 0.45 ± 0.03) (Fig. 1A). The logged ratio of ants to aphids declined significantly with increasing log of aphid count per colony (Fig. 1B, $F = 19.95$, 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 17757 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: 4 coccinellid larvae, 6 syrphid larvae, 4 syrphid pupae and 1 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.

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. 2; Mann-Whitney tests with Bonferroni correction). In the greenhouse experiment, as in the field, tending intensity was most commonly between 0 and 1 ant per aphid, with occasional instances of 1 to 2 ants per aphid. The mean ant-to-aphid ratio in the four largest treatments was 0.36 ± 0.08 (cf. Fig. 1A). 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. 2).

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. 3A-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. 3C-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 Figs. 3A-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. 4; $F = 6.83$, d.f. = 2, 45, $P = 0.0026$, $R^2 = 0.23$) whose AIC value, -184.4, is smaller than -180.5 for

- 1 the linear model. The nonlinear relationship indicates an increasingly negative relationship
- 2 between r and tending intensity with higher levels of tending.
- 3

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 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 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. 3C). 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. 1B, 3). 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 which occur occasionally in small aggregations (Fig. 1B), 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

1 and survivorship to adult (Table 1, Fig. 3A). Tending had no effect on per capita birth rate or
2 developmental time to maturity (Table 1). We do not have a mechanistic explanation for why *C.*
3 *populicola* suffered a survivorship rather than reproductive cost to tending; however, aphids are
4 clonal organisms selected for rapid and prolific reproduction rather than individual survival.
5 Further, tending by ants can take a physiological toll on honeydew-producing insects. Studies
6 have shown that honeydew production rate and composition can be affected by stimulation from
7 tending ants (Takeda, Kinomura & Sakurai 1982; Yao & Akimoto 2001, 2002; Katayama &
8 Suzuki 2002, Stadler & Dixon 2005). Increasing honeydew production may reduce an aphid's
9 assimilation efficiency or increase stress as a result of the high physiological demands of
10 ingestion and excretion during attendance (Stadler & Dixon 1998; Yao & Akimoto 2001). The
11 proportion of oligosaccharides (e.g., melezitose) in honeydew preferred by ants (Kiss 1981;
12 Volkl *et al.* 1999; Woodring *et al.* 2004) can be high in ant-tended *Chaitophorus* spp. (Fischer &
13 Shingleton 2001). Such sugars are likely costly to produce, as production of melezitose is
14 reported to decrease in *Chaitophorus* when not tended (Fischer & Shingleton 2001). A negative
15 direct effect of tending has been demonstrated in a few other aphid species, although the costs
16 were incurred in reproduction or developmental parameters rather than survivorship (Stadler &
17 Dixon 1998; Yao, Shibao & Akimoto 2000).

18 Reduced survivorship in our study is unlikely to result from either predation on the aphid
19 by the Argentine ant or disturbance of aphids by ants, for several reasons. First, predation on *C.*
20 *populicola* by the Argentine ant and aphid drop from the plant were rarely observed in the field
21 or greenhouse. Second, killed crickets provided a source of protein *ad libitum* throughout the
22 experiment, and no alternative sources of carbohydrates other than from aphid honeydew were
23 made available. The Argentine ant has a high demand for carbohydrates (Grover *et al.* 2007; Kay

1 *et al.* 2010). Without alternative sources of carbohydrates in the experimental arenas (Offenberg
2 2001), the Argentine ant is more likely to have utilized aphids as carbohydrate rather than protein
3 sources. Third, dramatic declines in aphid numbers (i.e., ≥ 5 individuals dying in one day), which
4 might be expected in pots where colonies switched from tending to predation, occurred in only
5 three replicates, one each from the 0, 100, and 500 initial worker treatments. Finally, previous
6 experimental work with various aphid species has shown that the strongly myrmecophilous *C.*
7 *populicola* does not fall off plants when exposed to alarm pheromones typically released by a
8 variety of aphid species when disturbed (Nault et al. 1976). Thus, neither ant activity nor manual
9 removal of F2 nymphs is likely to have caused disturbance to aphid colonies leading to
10 significant aphid drop from plants, consistent with our observations.

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

1 to harassment from ants (Bartlett 1961; Banks 1962; Herbert & Horn 2008). Another potential
2 benefit is sanitation service, which prevents the drowning of aphids in honeydew when aphid
3 densities are high (Way 1954; Wimp & Whitham 2001). Finally, untended aggregations were
4 rare, and, on average, smaller than tended aggregations. It is unclear without further
5 experimentation whether aphids cannot survive in the absence of ant protection (i.e., the aphid is
6 an obligate myrmecophile) or if the Argentine ant is simply very efficient at finding and
7 recruiting to aggregations of *C. populicola*.

8 Interestingly, larger aggregations of aphids and membracids typically recruit a
9 diminishing per capita number of tending ants (Fig. 1B; see also Addicott 1979; Cushman &
10 Whitham 1991; Breton & Addicott 1992; Morales 2000b; Bluthgen, Mezger & Linsenmair 2006;
11 Gove & Rico-Gray 2006; Harmon & Andow 2007). This negative density-dependence in per
12 capita ant recruitment means that small aggregations of *C. populicola* experience the greatest
13 levels of tending intensity (Fig. 1B), which can result in lower intrinsic rate of increase (Fig. 4)
14 relative to larger aggregations with lower per capita tending. Whether this positive density-
15 dependence in r leads to positive density-dependence in realized aphid population growth rate
16 will largely depend on how protective service by ants varies with tending intensity. If the level of
17 protection tends to decline with decreasing tending intensity, then mortality risk in aphids from
18 natural enemies could be positively density-dependent. Subsequently, the net effect of tending on
19 aphid population growth rate may be density-independent or negatively density-dependent,
20 depending on the relative magnitudes of direct (physiological) and indirect (protective) effects of
21 tending on the aphid. Only by understanding the full range of gross benefits and costs can one
22 begin to predict net effects of one partner on the other in these widespread mutualisms.

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6

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

2

3 Response	Error	Explanatory						
4 Variable	Distribution	Variables	Estimate	SE	χ^2	d.f.	<i>P</i>	
5 Survivorship	Binomial	Ant:aphid ratio ^a	-1.3445	1.1586	4.82	1, 32	0.0281	
6 to adult		Ant site	---	---	5.48	7, 32	0.6021	
7		Ratio*ant site	---	---	1.44	7, 32	0.9843	
8 Mean per capita	Poisson	Ant:aphid ratio	-0.1820	0.1660	1.82	1, 32	0.1775	
9 reproduction		Ant site	---	---	4.15	7, 32	0.7618	
10		Ratio*ant site	---	---	23.22	7, 32	0.0016	
11 Time to first	Poisson	Ant:aphid ratio ^b	0.0794	0.1567	0.69	1, 32	0.4076	
12 reproduction		Ant site	---	---	1.88	7, 32	0.9661	
13		Ratio*ant site	---	---	10.96	7, 32	0.1403	
14 Longevity	Poisson	Ant:aphid ratio	-0.5240	0.3476	9.48	1, 32	0.0021	
15		Ant site	---	---	5.29	7, 32	0.6247	
16		Ratio*ant site	---	---	1.32	7, 32	0.9879	

17

1 Table 1, continued.

2	Response	Error	Explanatory					
3	Variable	Distribution	Variables	Estimate	SE	χ^2	d.f.	<i>P</i>
4	Net reproductive	Poisson	Ant:aphid ratio	-1.3400	0.7214	9.95	1, 32	0.0016
5	rate, R_0		Ant site	---	---	7.24	7, 32	0.4041
6			Ratio*ant site	---	---	1.70	7, 32	0.9744
7	Generation	Poisson	Ant:aphid ratio	-0.1605	0.1161	12.27	1, 32	0.0005
8	time, G		Ant site	---	---	15.14	7, 32	0.0343
9			Ratio*ant site	---	---	12.77	7, 32	0.0778

10 ^a Mean ant:aphid ratio was calculated for the period from Day 0 to the date of 100% maturity in each replicate.

11 ^b Mean ant:aphid ratio was calculated for the period from Day 0 to the date of first reproduction in each replicate.

12

Figure legends

Plate I. 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.

Fig. 1. 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 - 2 September 2008 (least squares fit through logged data: $y = -0.31x + 0.01$).

Fig. 2. 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.

Fig. 3. 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.

Fig. 4. 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: $y = 0.136 + 0.027x - 0.042x^2$.

Plate I

Figure 1

Figure 2

Figure 3

Figure 4