

A balanced diet: Effects of ant (Hymenoptera: Formicidae) nutritional state on the balance between mutualism and predation upon aphids (Hemiptera: Aphididae)

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Abstract

Mutualisms are commonly dependent on the ecological context in which partners interact. In ant-aphid interactions, the dietary status of ants has been hypothesized to be one factor that may mediate ant-aphid interactions. This hypothesis was supported in one laboratory study with one ant species. We build upon that work here by conducting field trials involving three ant species. Boxed colonies of *Formica podzolica* (Francoeur), *Tapinoma sessile* (Wheeler), and *Myrmica incompleta* (Provancher) were provided either a tuna in oil or honey solution diet treatment to examine the effects of ant nutritional state on the mutualism between these ants and field colonies of the aphid *Aphis valerianae* (Cowen) feeding on *Valeriana edulis* (Nutt. Ex Torr. & A. Gray). A significant difference in tending rate and overall activity between treatments was seen in *F. podzolica*, with tuna-fed colonies tending but honey-fed ants remaining relatively inactive. Exploration outside of the nest box was uncommon for *T. sessile* and not observed for *M. incompleta*. In addition to these interactions, encounters between the aphid *A. valerianae* and the ants *F. podzolica* and *M. incompleta* were staged by moving ants to aphid colonies. A significantly different treatment effect was seen in *F. podzolica* consistent with the patterns seen in the unstaged interactions, except for the predation of one aphid in the staged interactions. No significant treatment effect was seen in *M. incompleta* staged interactions. *F. podzolica* tended aphid colonies did not appear to be protected from natural enemies, and aphid colonies suffered a marginally significant population decline when tended by tuna-fed ants. These results provide the field evidence that ant diet influences ant-aphid interactions.

Key Words

Mutualism, *Formica podzolica*, *Tapinoma sessile*, *Myrmica incompleta*, *Aphis valerianae*, *nutritional state*, *ant behavior*

Introduction

In mutualistic associations between species, such as those between ants and aphids, costs are borne and benefits reaped by both partners. From the perspective of aphid-tending ants, the aphids are exploited for their sugar-rich honeydew, used as nutriment by the ants. Myrmecophilous aphids in turn exploit their attendant ants for protection from predators, parasitoids, competitors, and problems associated with honeydew accumulation (Way 1963). Viewing the benefits of these associations, some have suggested that mutualisms are best viewed as a mutual exploitation where each partner receives a net benefit (Conner 1995; Herre et al. 1999). This exploitation, however, carries costs. Bronstein (2001) outlined how costs (including investment in reward production, damage by mutualists, exploitation by non-mutualists, dependence on mutualist partners, and loss of evolutionary flexibility) may shape mutualisms in addition to their benefits. Furthermore, Bronstein (1994) argued that interactions that are typically considered mutualisms are better considered as interactions along a continuum from mutually beneficial (+,+) to neutral (0,0) to exploitative (+,-).

Selective pressure favors mutualists that derive the maximal benefits from their partners; therefore, mutualistic associations are subject to shift to predation and parasitism (Sachs and Simms 2006). The evolutionary history of ants and aphids is estimated to include multiple events of emergence and decay of mutualistic associations (Bristow 1991; Shingleton and Stern 2002). Many studies have documented the predation on myrmecophilous aphids by tending ants (Pontin 1958; Skinner and Whittaker 1981; Sakata 1994, 1995; Novgorodova 2005; Billick et al. 2007). The degree to which interactions between ants and trophobiont aphids are

mutualistic or antagonistic depends heavily on many aspects of the ecological context of the interaction (Cushman and Addicott 1991; Bristow 1991). Where a potential ant-aphid mutualism falls in the space between true mutualism and antagonism has been demonstrated to depend on several factors including competition (Cushman and Addicott 1989), host plant quality (Breton and Addicott 1992a; Mooney and Agrawal 2008), and presence of natural enemies (Yao et al. 2000).

Many mutualisms between ants and aphids are facultative (Stadler and Dixon 1998), which allows each mutualist the flexibility to abandon its partner in the event of unfavorable exploitation (Sachs and Simms 2006). Several studies of mutualist systems suggest that predatory and parasitic interactions can evolve into mutualisms (Crepet et al. 1991; Stadler and Dixon 1999; Billick et al. 2007) and characterize the resulting associations following mutualism decay (Sachs and Simms 2006). Examination of the mechanisms underlying the tipping point between mutualism and predation is warranted to understand the evolutionary origin and maintenance of the mutualist life-history strategy.

Several dietary conditions of the ant colony have been suggested to influence ant abandonment of mutualism in favor of predation. They include the quantity and quality of honeydew reward (Sakata 1995; Völkl et al. 1999), and nutritional state of the ants (Way 1954; Rosengren and Sundström 1991; Offenberg 2001). Sakata (1995) suggests that *Lasius* ants exhibit more predatory behavior towards aphid species that offer relatively less honeydew. Offenberg (2001) observed an eight-fold increase in predation by *Lasius niger* L. on *Aphis fabae* Scop. when the ants were provided honey solution *ad libitum* in the laboratory; ants increased

predation on aphids only when the ant's carbohydrate needs were met, but did not increase predation on aphids in response to a lack of protein in their diet. As larvae and queens are the only individuals that consume large amounts of protein, Offenberg (2001) suggests that carbohydrates (sugars) are required for colony maintenance while protein is devoted to colony growth. Aphid responses to natural enemies and host-plant differences have been suggested to influence the behavior of tending ants (Nault et al. 1976; Cushman 1991), which could impact the net balance of costs and benefits for both the ant and the aphid.

This study seeks to build upon Offenberg's (2001) by studying the effects of ant nutrition on interactions with aphids in a field setting. This study also expands upon Offenberg's (2001) work by testing this hypothesis with three ant species, representing three ant subfamilies that differ in their evolutionary history of association with aphids. The applicability of Offenberg's (2001) conclusions to broader ecological contexts are limited by the fact that the study was conducted in a laboratory in the absence of climatic variation, natural plant quality variation, other herbivores, and aphid enemies that may influence ant and aphid behavior. Finally, in order to truly understand what, if any, indirect effects ant diet has the outcome of ant-aphid interactions in terms of aphid performance, this study must be conducted in a field setting.

This study characterizes and compares the behavioral responses of independently evolved mutualist strategies in three species of ants to dietary supplementation and restriction in a field setting. Specifically, we ask three questions: **1) Are the differences in tending rate and shifts to predation reported by Offenberg (2001) observed in a field setting?, 2) How do three ant species from different subfamilies, ecologies, and evolutionary origins of mutualism**

with honeydew producers compare in their responses to myrmecophilous aphids when under varying dietary treatments?, and 3) What are the indirect effects of ant colony nutritional status on aphid performance?

Methods

Study Site

This study was conducted approximately 1 km NNW the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Gunnison County, Colorado (approximately 38° 57' 33" N, 106° 59' 21" W). The elevation of the collection and study sites ranged between 2,850-3,000 m. Spatial data (Global Positioning System points) are available at <http://www.rmbl.org>. See Langenheim (1962) for a description of local plant communities. Much attention has been directed towards ant-hemipteran interactions at the RMBL. Several widely cited studies on ant-aphid mutualisms have been conducted in the area (Addicott 1978; Cushman and Addicott 1989; Breton and Addicott 1992b), as have many more recent studies on ant-aphid and ant-membracid mutualist systems (e.g. Billick and Tonkel 2003; Reithel 2003; Billick et al. 2007; Reithel and Billick 2006).

Study Organisms

Three aphid-tending ant species (*Formica podzolica* Francoeur, *Tapinoma sessile* Wheeler, and *Myrmica incompleta* Provancher) were used in this study. *Formica podzolica* is a polygynous, omnivorous, mound-building ant in the subfamily Formicinae (Deslippe and Savolainen 1995). Colonies may be either monodomous, that is, contained in a single nest mound or polydomous, with multiple nest mounds (DeHeer and Herbers 2004). *Tapinoma*

sessile is an omnivorous predator/scavenger in the Dolichoderinae subfamily that nests under shallow rocks, readily dispersing in response to disturbances (Smallwood 1982; WKP, personal observation). *Tapinoma sessile* may be monogynous (one queen) or polygynous (up to 200 queens; Smith 1928). *Myrmica incompleta* is an ant species in the subfamily Myrmicinae that typically nests in montane meadows and aspen forests (Gregg 1963). All three species are commonly found in the study meadow. Foraging workers of *T. sessile* are typically subordinate to those of *Formica* spp. and *Myrmica* spp., and must find and collect food before more dominant species displace them (Fellers 1987). The worker caste of ants in these three subfamilies of Formicidae (Formicinae, Dolichoderinae, and Myrmicinae, respectively) has independently evolved abilities to collect and transport honeydew (Stadler and Dixon 2005). Voucher specimens of *M. incompleta* have been deposited at the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA.

The aphid species selected for this ant-aphid interaction was an abundant aphid, provisionally identified as *Aphis valerianae* Cowen, which feeds on the leaves, stems, and inflorescences of *Valeriana edulis* Nutt. Ex Torr. & A. Gray. Palmer (1952) reported collecting apterous vivipara of *A. valerianae* between 30 June and 6 September. The over-wintering biology of this rarely studied species remains unstudied. At least five ant species (*T. sessile*, *F. podzolica*, *F. lasioides*, *Myrmica discontinua*, and *Camponotus* sp.) have been observed tending *A. valerianae* in the study meadow, though not all aphid colonies are tended (WKP, personal observation). Voucher specimens of the aphids will be deposited at the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA and sent away for identification confirmation.

Experimental Design

For each ant species, five colonies were excavated between late-June and mid-July and divided into experimental colonies (=divisions of the original source colony). Experimental colonies approximated the species-specific proportions of workers to brood observed in source colonies. *Formica podzolica* experimental colonies consisted of 200 workers and 40 larvae; *Myrmica incompleta* experimental colonies consisted of one queen, 200 workers, and 50 larvae; *T. sessile* experimental colonies consisted of one queen, 100 workers, and 10 larvae. Two experimental colonies from each source colony were separated and each was provided one of two diet treatments (i.e. one source colony yielded two experimental colonies, one for each diet treatment). Source colonies of *F. podzolica* rarely contained queens (i.e. colonies at this locality are polydomic; WKP, personal observation), which justifies the lack of a queen in experimental colonies. Experimental colonies of *F. podzolica* and *M. incompleta* were maintained in 26x35x17 cm opaque plastic containers rimmed with Tree Tanglefoot Pest Barrier (Tanglefoot Company, Grand Rapids, Michigan) in soil collected near the original nest and a large flat rock (*F. podzolica*) or wooden mulch (*M. incompleta*) as nesting materials. The container lids were propped open on one side with a 15-20 cm length of wire to prevent flooding and desiccation. Experimental colonies of *T. sessile* were held in 946 ml volume (4 US cups) cubic, clear plastic containers filled with approximately 5 cm of soil and a small flat rock as nesting materials. A hole was cut in the center of the lid to allow the passage of the wire bridge (described below), and the lid was coated with Fluon (AGC Chemicals America, Inc., Exton, Pennsylvania) to prevent escape. This was then placed in a larger container (identical to those

described above) to prevent flooding and desiccation. These assemblies will henceforth be referred to as nest boxes. All treatments were regularly misted with water throughout the study to prevent desiccation. Prior to field deployment, nest boxes were maintained on the premises of the RMBL under a WeatherPORT® (WeatherPORT Corp., Delta, Colorado, USA).

Approximately 72 hours before field deployment, experimental colonies were provided one of two treatment diets, 1) honey solution alone (10% carbohydrate by mass) or 2) tuna alone (25-30% protein, 8-10% fat by mass). Food items were provided in 15 mL plastic culture tubes and were replenished frequently to avoid spoilage. Nest boxes were moved to aphid-bearing plant stems after this period and allowed to settle before observation. A wire bridge, secured to a rock or piece of wood in the nest box, was tied to the aphid-bearing valerian stems to allow for exploration and recruitment of foraging ants from the experimental colony. The plant stems, each bearing approximately 40-75 aphids, with Tree Tanglefoot Pest Barrier applied to the base, separated from the stem by a ring of tape, to prevent access to nutrition outside of that provided by the aphids and the diet treatment. Additionally, plant stems were stabilized by securing them to bamboo stakes approximately 90 cm in length with wire twist ties. Five replicates per treatment per ant species (= trials) were conducted using naïve colonies, aphids, and plant stems for a total of 30 trials (3 ant species x 2 diet treatments x 5 trials).

Observations of ant tending, scouting, defensive, flower visiting, and predatory behavior were collected between 23 July 2009 and 3 August 2009. Snapshot observations were made, each separated by a minimum of one hour and comprising of a fifteen second per experimental

colony census of ant, predator, parasitoid, and herbivore activity and abundance. Also, focal observations were made on tending ants to characterize colony time allocation. Individual activity (tending, scouting, defensive posturing, flower visiting, and predatory behavior) of each ant on the plant was recorded every minute during the focal observation of an experimental colony. Experimental colonies for focal observation were identified during and conducted in between snapshot observations. Colonies were selected for focal observation opportunistically; only colonies with active ants were observed in this detail.

After the experiment, the number of workers and brood were re-counted to account for variation in observed behavior and to standardize each experimental colony's nutritional requirement (accounting for differential mortality, loss of workers due to falling off bridge/plant, and fecundity of queens across experimental colonies). Differences in demography between treatment pairs could account for different behavioral patterns of the ants. This post-experiment census controls for these differences.

Statistical Analysis

Source colonies are assumed to be independent of each other. Experimental colonies derived from the same source colony, however, were not assumed to be independent. Rather, this division allows for accounting for variation among source colonies by comparing treatment differences between the pair of experimental colonies derived from the same source colony. Fisher's Exact Test was used to test for treatment effects within a species. Data were analyzed using the SPSS 16.0 (SPSS, Inc., Chicago, Illinois, USA) statistical package.

Results

Fifty-two snapshot observations of each colony and total of 811 minutes of focal observations were made on six days between 23 July 2009 and 3 August 2009. Four *F. podzolica* and two *T. sessile* experimental colonies were observed tending *A. valerianae*. No *Myrmica incompleta* experimental colonies were observed tending and no instances of predation were observed by any species of ant.

Of the four tending *F. podzolica* experimental colonies, all were under the tuna diet treatment. The other six *F. podzolica* colonies (one tuna treatment and all five honey treatments) were not observed tending aphids (Fig. 1). A Fisher's Exact Test revealed a significant difference between diet treatments ($P = 0.048$). Of the tending *T. sessile* colonies, one was under the tuna treatment and one was under the honey treatment. *Myrmica incompleta* was never observed on the *V. edulis* stems. No analysis of treatment effect was performed for *T. sessile* and *Myrmica incompleta*

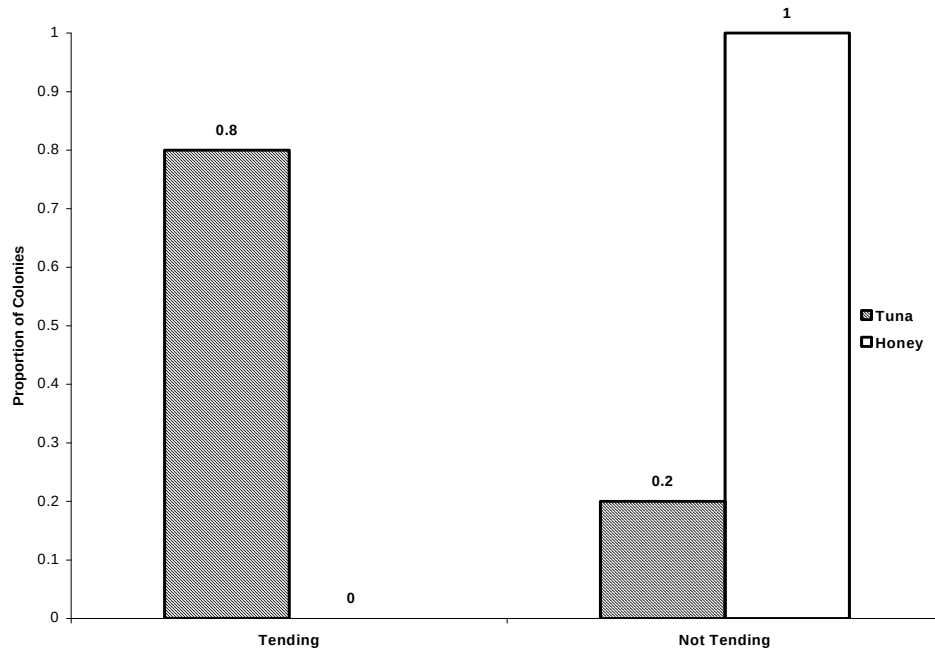


Fig. 1. The proportion of *Formica podzolica* colonies tending *Aphis valerianae*, for each of the two diet treatments.

Formica podzolica activity on *V. edulis* stems was not limited to the tending of aphids (defined as palpating aphids with the antennae and/or collecting honeydew). Scouting (moving around the plant stem) and flower visitation (collection of nectar from flowers) were also observed (Fig. 2).

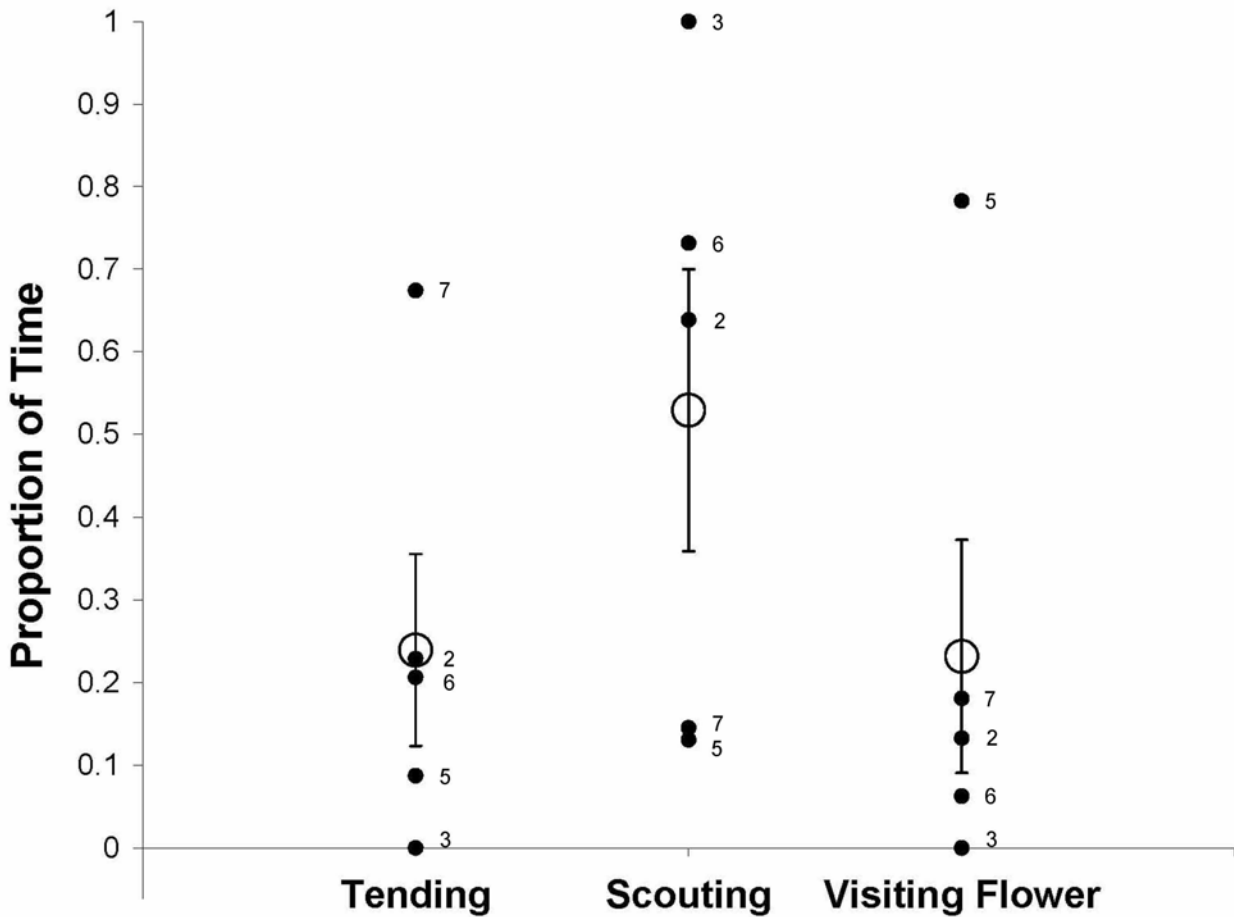


Fig. 2. The means (large open circles) $\pm$ 1 standard error of tending, scouting, and flower visitation behaviors by *Formica podzolica* under tuna diet treatment, expressed as a proportion of time. Small solid circles denote means of individual experimental colonies (e.g. 3 was the only experimental colony that did not tend aphids). Numbers correspond to spatial metadata available at <http://www.rmbi.org>.

Over 811 minutes of focal observation, the mean proportions of time tending, scouting, and visiting flowers for *F. podzolica* under tuna diet treatment were 0.240, 0.529, and 0.232,

respectively. Variation among experimental colonies was seen: the proportional time tending ranged from 0-0.674; the proportional time scouting ranged from 0.130-1.00; the proportional time visiting flowers ranged from 0-0.783. This variation is not exclusively attributable to ant colony effect, but rather is likely due to a combination of ant colony, aphid colony, and plant effects. No predation upon aphids by ants was observed during focal observations.

In addition to not tending, *M. incompleta* was not observed outside of the nest box. Likewise, for *T. sessile*, except for one worker that was observed outside of the nest box of one of the colonies (during three snapshots across two days). No comparisons of foraging behavior between treatments or species were made due to the lack of active ants on the plant stem. Within the nest boxes of both species, workers could be seen excavating tunnels and foraging at the diet treatment tubes.

Interactions between *F. podzolica* and *M. incompleta* ants and the aphid *A. valerianae* were staged by moving a single worker from the nest box to the aphids with a grass stem on two different days. On the first day, all five tuna-fed *F. podzolica* workers immediately tended the aphids, while all five honey-fed workers ignored the aphids. No predation events were observed. More behavioral variation was observed on the second day, where tuna-fed colonies tended (3 experimental colonies), ignored (1 experimental colony), and preyed upon (1 experimental colony) aphids. Honey-fed colony behavior remained more constant, as 1 tended and 2 ignored aphids (2 experimental colonies were lost to flooding between observations). These observations demonstrate a significant treatment effect between experimental colonies (Fisher's exact; $P = 0.003$) and are consistent with the patterns of tending seen when the

interactions were not staged (Fig. 3). Diet treatment did not produce any significant differences in *M. incompleta* behavior ($P = 0.133$). Experimental colonies of both treatments ignored and tended aphids, though only tuna-fed experimental colonies tended.

Aphid population dynamics were monitored for all colonies between 22 July 2009 and 10 August 2009. A biologically significant ($t_{\text{paired}} = 2.324$; $df = 4$; $P = 0.081$) treatment effect was seen between aphid colonies attached to *F. podzolica* experimental colonies. The mean per capita growth rates over the 19-day period for honey-fed and tuna-fed colonies were 1.57 and 0.60, respectively, resulting in growth for untended colonies and decline in tended colonies. No significant treatment effects on aphid per capita growth rate were seen in *M. incompleta* ($t_{\text{paired}} = 0.921$; $df = 4$; $P = 0.409$) and *T. sessile* ($t_{\text{paired}} = -1.075$; $df = 4$; $P = 0.343$).

Discussion

These data indicate that ant behavior towards aphids is conditional upon the dietary status of the ant colony. When provided tuna in oil, *F. podzolica* tended aphids, though this interaction had a marginally significant negative impact on aphid population growth. When provided honey solution, this ant ignored aphids and rarely was observed on plant stems. We believe that the artificial nest boxes dissuaded *M. incompleta* and *T. sessile* ants from foraging on the plant, making it impossible to test for differences between diet treatments with unstaged encounters. However, the staged encounters between both *F. podzolica* and *M. incompleta* showed results that were parallel to those for unstaged encounters for *F. podzolica*, suggesting that these conclusions are generally valid for both ants.

Though a different ant-aphid system, our results partially support those of Offenberg (2001), in that protein-fed ants tended more frequently than honey-fed ants. However, where Offenberg found predation from protein-starved *Lasius niger*, no predation events were observed for *F. podzolica* in our study. Additionally, our results differ from those of Del-Claro and Oliveira (1993) who report continued ant-aphid interaction in the presence of additional sugar resources.

The negative effect of tending was not anticipated in this previously unstudied system and suggests that the interaction between *F. podzolica* and *A. valerianae* was not mutualistic. If ant attendance has a negative effect on aphid fitness, this could imply exploitation on the part of the ants. Yao et al. (2000) demonstrated the phenotypic plasticity of aphid investment in honeydew rewards by rearing aphids with and without ant attendance. Tended aphids were smaller in size and produced fewer embryos than untended aphids, indicating that the physiological demands of producing attractive honeydew rewards has a direct negative impact on fitness. Also unexpected was the rarity of predation or parasitism events by arthropods other than ants. *Valeriana* species, including *V. edulis*, are known to produce iridoid glycosides, a class of plant defense secondary metabolites (Castillo et al. 2002; Williams and Bowers 1987). Given the few predators observed, we speculate that *A. valerianae* chemically defend themselves by sequestering iridoid glycosides in a fashion similar to that previously documented in several lepidopteran families (Bowers and Puttick 1982; Gardner and Stermitz 1988; Boros et al. 1991; Stermitz et al. 1988), chrysomelid beetles (Willinger and Dobler 2001), and the aphid *Acyrtosiphon nipponicus* (Nishida and Fukami 1989). It may be that the aphids

in our study did not require ant protection, but still paid the physiological, and subsequently fitness, cost.

In addition to affecting aphids, ant diet may have consequences for the plant. Tuna-fed *F. podzolica* spent more time visiting flowers of *V. edulis* than honey-fed *F. podzolica*. This could have three contrasting influences from the point of view of the plant: 1) ants benefit the plant by providing pollination services (rare, see Peakall et al. [1991] for commentary), 2) ants are detrimental to the plant by deterring pollinators (ants were observed disturbing pollinating flies; see Ness [2006] for empirical evidence), and 3) ants benefit the plant by removing untended herbivores (Styrsky and Eubanks 2007). If these potential costs and benefits result in a net benefit for the plant, the mutualism in this system could be between ants and plants rather than ants and aphids.

A fourth beneficial influence to the plant could be distraction of ants. Tuna-fed *F. podzolica* spent equal time tending aphids as visiting flowers and collecting nectar, which could imply that the plant is able to partially distract ants from tending aphids. Though multiple previous studies have found extrafloral nectaries, at least partially, distract hemipteran-tending ants (Becerra and Venable 1989; Bentley 1977; Rico-Gray and Morais 2006), this theory is debated (Del-Claro and Oliviera 1993; Fiala 1990). To our knowledge no work has demonstrated floral nectaries as mediators of the ant-hemipteran mutualism. As with all potentially mutualistic interactions, the balance of costs and benefits for each partner will determine the net outcome of the interaction.

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