**Formica ant and Cinara aphid mutualisms on limber pines (Pinus flexilis)**

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**ABSTRACT**

Ant-aphid mutualisms play important ecological roles in many ecosystems. While *Formica* ants are well studied model organisms, pine-specialist *Cinara* aphids remain understudied. Moreover, there is little research on ant-aphid mutualism in subalpine ecosystems. We investigated multiple species of *Formica* ants and their mutualistic interactions with *Cinara* aphids on the limber pine *Pinus flexilis* in a subalpine ecosystem. *Cinara apini* colonies were found tended to by silver, black, and red *Formica* ants, and they occupy older branches and trunks of the pines. A different, unknown *Cinara* species forms species-specific mutualisms with the brown *Formica* ant, occupy new growth twigs, and are more aggressively defended by brown ants. All ants forming mutualist colonies with aphids exhibited little modification in behavior and defense in the presence of sugar bait. The results of our study give insight into several *Formica* and *Cinara* life histories as they relate to ant-aphid mutualisms in a subalpine ecosystem.

Keywords: ant, aphid, mutualism, subalpine

**INTRODUCTION**

Mutualisms are a common form of species interaction, in which both species gain a net fitness benefit. Species involved in a mutualism may gain resources that would otherwise be difficult to acquire, or receive protection from predators and parasites. One such mutualism is the relationship between ants (family Formicidae) and aphids (superfamily Aphidoidea), in which ants defend aphids from predation in exchange for honeydew. Aphids are gregarious phloem-feeders which reproduce in vast numbers, thus acting as predators of many plant species, and prey of many animals (Ives 1993). After feeding, they secrete a sugary waste called honeydew. Ants will guard aphid patches to consume honeydew, and in the process, remove waste from aphid aggregations and protect aphids from predation (Mooney 2005). Honeydew can be a vital source of sugar for ants, especially when fruits and nectar are not abundant (Mooney 2005). Aphids experience very high predation pressure, and thus may benefit greatly from ant protection, especially when their population numbers are low (Breton 2005). In this way, ant-aphid mutualisms provide considerable advantages to both taxa.
Mutualistic relations with aphids can allow ants to thrive in food-limited environments, thereby expanding the range and impact of these ants. Ant-aphid mutualism can also have varying consequences for host plant species. Host plants may benefit via presence of ant guards—in the presence of ant-aphid mutualists the majority of plants benefit by experiencing reduced damage, increased growth, and increased reproduction (Styrsky 2007). Ant-aphid mutualism can also harm plants, as Kilpeläinen et al found that *Formica rufa* and *Cinara* aphids mutualism decreased 30-year-old Norway spruce (pine family) growth by 7.3% but had no effect on other age standings. Alternatively, ant-aphid mutualisms on host plants can have relatively negligible effects; pine growth will sometimes go unaffected by ant-aphid interactions (Kilpeläinen 2009). These previous studies exemplify the interspecific variation that exists within ant-aphid mutualisms.

Among aphid species, there is considerable variation in interspecific associations and aphid microhabitat preferences. For example, pine-specialist *Cinara* are known to form associations with *Formica* wood ants as well as *Lasius* ants (Akyürek 2016). *Cinara* exploit various microhabitats among their pine hosts: *C. brauni* feeds on current year and one year-old growth of *Pinus nigra* (Massuti 2005), *C. piceae* on older branches and trunks of spruce (*Picea*) trees (Blackman 2000), and *C. piceicola* on spruce (*Picea*) needle bases and shoots (Binazzi 2009). Despite this research, the interspecies associations and microhabitat preferences of many species remains unknown.

Aphid colony defense by ants has been extensively studied - studies indicate that when ants are tending to a larger colony of aphids, they play a less significant role in aphid defense (Breton 1995). Moreover, ants may decrease tending behavior or cease tending aphids all together when exposed to a more preferable source of sugar (Sakata 1995). However, this varies by sugar content and concentration, and previous studies have indicated diminished interest of ants for sucrose bait during the summer months (Sudd 1985).

Ant-aphid mutualisms are geographically widespread (Way 1963) and well-researched amongst crops and temperate and tropical ecosystems (Styrsky 2007), but relatively understudied within subalpine ecosystems and amongst pine-feeding *Cinara* aphids. There has been no previous research documentation of ant associations and microhabitat preferences of any known aphid species found on the subalpine-dwelling limber pine *Pinus flexilis*. The three documented *Cinara* aphid species on *P. flexilis* have been understudied in their natural history and in their interactions with ants. However, such mutualisms are important to research because ants have large ecological impacts on subalpine habitats. For example, *Formica rufa* colony mounds were linked to forest tree species composition, slope aspect, and canopy closure (Risch 2005).

In order to expand our understanding of *Formica* ants and *Cinara* aphids in a subalpine setting, we studied these ant-aphid mutualists on limber pines (*Pinus flexilis*) in the White Mountains of California. In our observational study, we noted species and microhabitat trends of ant-aphid associations. We hypothesized...
that ant-aphid associations would not be species-specific, and that there will be interspecific differences in aphid microhabitat preferences. We hypothesized that aphid to ant ratios will be negatively correlated with defensive behavior of ants. We also hypothesized that the presence of honey would have little effect on aphid to ant ratio and defensive behavior. We were motivated to expand the knowledge on ant-aphid mutualism, as well as the natural history of *Cinara* aphids on *P. flexilis*.

**METHODS**

**2.1 Study Site**

Our study was conducted near Crooked Creek Field Station, at the University of California White Mountain Research Center in the White Mountains at the Inyo National Forest. We studied interactions amongst four *Formica* ant species and two *Cinara* aphid species on *Pinus flexilis* trees from August 2–5, 2018. One aphid species, the “grey aphid,” was identified as *Cinara apini*. The other species could not be identified, and will be referred to as the “red aphid” based on coloration and morphology. The *Formica* species could not be identified beyond genus, and will be referred to as “silver ant,” “brown ant,” “black ant,” and “red ant.” Samples were collected of each *Formica* and *Cinara* morphospecies.

**2.2 Observational Study**

We examined *P. flexilis* trees within the Crooked Creek station for ant-aphid patches. Patches initially featured at least one aphid being visibly attended by ants. Ant-aphid mutualistic patches and their host trees were marked and numbered. For each patch, we recorded ant and aphid species identity, and ant and aphid numbers. We classified the microhabitat of the patch as either the main trunk and branches, or the needle bases. Tagged patches were revisited two more times at approximately 24 hour intervals to see if patches remained in their original location. Patches that were absent from their original site and patches that reappeared on following days were classified as mobile. To test defensive behavior, we prodded aphids within a patch with a 0.5mm wide pencil lead and counted the number of prods required to elicit a defensive response. Aphids were prodded up to 10 times at one second intervals. A defensive response was classified as at least one ant attempting to bite the pencil. Defensive behavior was classified into one of three categories: immediate response (1–2 prods), intermediate response (3–9 prods), and no response (no aggression after 10 prods).

**2.3 Manipulative Study**

For the manipulative test, we selected 10 sites for each of the four ant species, recording aphid and ant species composition and aphid and ant numbers prior to administering honey. We placed 5 mL of honey 1 cm away from each site, and the ants at the site were observed for 5 minutes. After 5 minutes, we counted the number of ants eating honey and the number of ants tending aphids. We also tested defensive behavior of the aphid-tending ants using the pencil prodding method.

**2.4 Data Analysis**

All data analysis was conducted using JMP 14 statistical software. We analyzed the
differences in ant and aphid taxonomy associations with a chi-square test. We examined whether ant or aphid abundance varied among species with ANOVAs. The effect of number of aphids on ants and ant to aphid ratio was tested with an ANOVA. The association between microhabitat type and aphid species was analyzed with a chi-square test. We used a chi-square test to investigate whether ant species differed in their defensive behavior. The effect of ant number, and aphid to ant ratio on defensive behavior was analyzed with logistic regressions. Differences in mobility of aphid clusters across time and species were analyzed with chi-square tests. To examine interspecific differences in response to honey, we compared the number of ants eating honey between species with an ANOVA. The effects of aphid to ant ratio on number of ants eating honey were also analyzed with an ANOVA. In order to examine the effect of honey treatment on defensive behavior, we derived one sample from observational study and one from our manipulative test, from both of which we excluded responses after 10 prods, and compared the difference in defensive behavior with a two-way ANOVA.

RESULTS

3.1 Ant-Aphid Association

Silver ants, red ants, and black ants largely associated with species of *C. apini*, whereas brown ants typically associated with red aphids (chi-square test: N = 145, $X^2 = 97.24$, $P < 0.01$, Figure 1). There was no dramatic difference in number of ants on a cluster among different ant species (ANOVA: N = 161, $F_{(3,157)} = 1.90$, $P = 0.13$). *C. apini* had marginally higher cluster sizes than red aphids (two-tailed t-test: N = 149, $T = 1.81$, $P = 0.07$). With greater number of aphids per cluster, number of ants increased (ANOVA: $R^2 = 0.49$, N = 153, $F_{(1,143)} = 146.17$, $P < 0.01$, Figure 2). However, ant to aphid ratio decreased as aphid cluster size got bigger (ANOVA: $R^2 = 0.10$, N = 147, $F_{(1,158)} = 17.54$, $P < 0.01$, Figure 3). Microhabitat types affect the colonized aphid species, with red aphids typically on needle bases and *C. apini* on branches (chi-square test: N = 159, $X^2 = 109.93$, $P < 0.01$, Figure 4).

Figure 1. Ant species associated with different aphid species. Red bar regions represent colonies of red aphids and grey bar regions represent colonies of *C. apini* (grey aphids). Brown ants largely associated with red aphids and other ants associated with *C. apini*.

Figure 2. Number of ants increased with aphid colony size. Black dots represent each cluster of aphids with ants. Blue line is line of best fit.
Figure 3. Ant aphid ratio decreases as aphid number increases. Black dots represent clusters of ants and aphids. Blue line is line of best fit.

Figure 4. Microhabitat affected the distribution of different aphid species. Red bar regions represent colonies of red aphids and grey bar regions represent colonies of C. apini (grey aphids). Red aphids tended to feed on limber pine tips whereas grey aphids tended to feed on branches.

3.2 Ant Defensive Behavior

Brown ants exhibited the most intense defensive behavior, but black ants least actively responded to our prods (chi-square test: N = 145, F(6) = 24.89, P < 0.01, Figure 5). The more ants on one cluster the more aggressive they were (logistic regression: N = 144, $X^2_{(2)} = 18.41$, P < 0.01, Figure 6).

However, aphid to ant ratio did not have an effect on defensive behavior (logistic regression: N = 145, $X^2_{(2)} = 1.33$, P = 0.51).

Figure 5. Ant species respond differently in defending aphid patches. Based on the number of prods required to trigger a bite, defensive response was categorized as immediate (1-2), intermediate (3-9), or no response (>9). The quicker an ant species reacted to our attack, the more aggressive they were. Brown ants were the most aggressive in defending their aphids and black ants defended least actively.

Figure 6. Defensive response became more rapid as number of attending ants per ant-aphid colony grew. Defensive response is more quickly enacted by tending ant colonies of larger sizes. Error bars represent ±1 standard error.
3.3 Aphid Mobility

From day 1 to day 2, the mobility of aphid clusters did not differ between two aphid species (chi-square test: N = 54, Χ²(3) = 0.74, P = 0.39), and it also did not differ among associated ant species (chi-square test: N = 54, Χ²(3) = 2.40, P = 0.49). From day 2 to day 3, however, the mobility of red aphid clusters was much lower than that of grey aphid clusters (chi-square test: N = 53, Χ²(3) = 6.35, P = 0.01, Figure 7). Clusters guarded by red and brown ants also moved more frequently (chi-square test: N = 51, Χ²(3) = 12.57, P < 0.01, Figure 8).

Figure 7. Aphid colonies in C. apini (grey) and red aphids persisted to differing extents. Red aphid colonies all persisted unmoved throughout the day 2 to day 3 period, whereas more C. apini colonies moved.

Figure 8. Mobility of ant-aphid clusters differed by Formica ant species. Brown and red Formica ants largely remain with aphid colonies over the observed day 2 to day 3 period, whereas some mobility was observed in the aphid colonies guarded by black and silver Formica ants.

3.4 Behavior in Presence of Food

More silver ants ate honey than brown ants (ANOVA: N = 40, F(3,36) = 3.42, P = 0.03, Figure 9). Aphid to ant ratio did not affect the number of ants eating honey (ANOVA: N = 40, F(1,38) = 0.05, P = 0.83). After the honey treatment, interspecific differences in defensive behavior did not change (chi-square test: N = 26, Χ²(6) = 12.85, P = 0.05, Figure 10). However, number of ants that remained on the clusters had no effect on defensive behavior (logistic regression: N = 26, Χ²(6) = 0.91, P = 0.63), so did aphid-ant ratio (logistic regression: N = 26, Χ²(6) = 12.85, P = 0.05).
DISCUSSION

4.1 Ant-Aphid Association

While silver ants, red ants, and black ants all associate with *C. apini*, brown ants predominantly associate with red aphids. Furthermore, the brown ant-red aphid patches were predominantly found on only one tree. Red aphids and *C. apini* differ in both microhabitat and associated ant species, which lends doubt to the notion that these two species are competing for space or ant care. Alternatively, the condition of the host plant may be discouraging *C. apini* and their associated ant species from settling. The tree in which we observed most of the brown ant-red aphid patches displayed many browning needles, and some species of aphids have been shown to flee and avoid host plants which are in poor health (Hough 1922). Conversely, the poor health of the tree may actively attract red aphids, as some species of aphids are known to prefer diseased host plants (Macias 1969). If sick trees are a limited resource for red aphids, this would explain why red aphids were uncommon on other trees, and concentrated on one sick tree (Pyke 1977). While brown ants are most frequently observed with red aphids, they have occasionally been observed with *C. apini*. Moreover, while silver ants, red ants, and black ants predominantly tend *C. apini*, they have also been observed with red aphids. This suggests that the ant-aphid associations we studied display trends in species and microhabitat, but can still occur outside of their preferred circumstances.
4.2 Defensive Behavior

Among the ant species, brown ants were most aggressive in defending their aphid clusters, while black ants were least aggressive. Brown ants may simply have the most aggressive archetype, which may also manifest in competitive exclusion of other ant species (Czechowski 2013), as previously speculated. Ant to aphid ratio had no effect on defensive behavior, which contradicted our initial hypothesis. A 1995 study by Breton et al. had shown that ants play a less proactive role in aphid tending when the ant to aphid ratio is lowered, but this did not appear to diminish defensive behavior in the ant-aphid mutualisms we studied.

4.3 Mobility of Ant-Aphid Patches

Brown ant-red aphid patches were observed being less mobile than other ant-aphid associations, perhaps because of brown ant aggression. Red aphids under careful protection by brown ants may experience reduced predation pressure, and this may reduce the need for patch movement. Alternatively, aphids may be under “stricter” control by brown ants via aphid wing amputation, chemical secretions inhibiting wing development (Kleinjan 1975) or chemical “fencing” (Oliver 2007).

Although C. apini is a “sedentary” aphid type (both C. apini and red aphids were largely wingless nymphs and apterae), mobility rates among C. apini patches were high. This may be due to high mobility in red, black, and silver ant species (Dixon 1958), as ants are known to herd their aphid towards better phloem sap extraction sites (Collins 2002). Alternatively, grey aphids may be more mobile in search of a particular resource (food, mates, oviposition target) or more prone to deserting as an escape response (Hough 1922). Likewise, as individual colony movement was not tracked, it is possible that the measure of mobility used may have been confounded by predation or involuntary weather-induced displacement. Rain and hail were experienced between the first and second observational periods, and aphids have been shown to disperse from host plants in the face of harsh weather (Mann 1995). However, aphid patch mobility was higher between the second and third observational periods, when the weather was mostly clear.

4.4 Behavior in Presence of Food

Most ant species readily took the honey bait, however the proportion that did varied amongst different ant species. Brown ants were least inclined to accept bait, and largely continued tending to their aphid colonies. This behavior along with elevated defensive tendencies in brown ants suggests different life history strategies in regards to aphid attendance in brown ants relative to other aphid-tending Formica species. Brown ants may be more reliant on their aphid mutualists and consequently more protective overall.

Similarly, Sudd et al. noted alternative food sources such as sucrose-water mixtures have limited acceptance by tending ant populations, especially from mid-spring throughout the summer months (Sudd 1985). Previous studies also indicate that the trisaccharide composition of honeydew produced by aphids is more desirable than monosaccharides or disaccharides (Völkl 1999)—primary components of the sucrose and honey baits used (White 1980). The lack
of change in ant defensive response in presence of honey bait also supports a lack of interest, indicating that honey is a lower-value food source than honeydew.

4.5 Significance and Future Studies

Our study examined ant-aphid mutualisms among Formica ants and the relatively understudied Cinara aphids. Ant-aphid mutualisms are ubiquitous around the world, yet they remain understudied outside of economically important plants and temperate and tropical ecosystems. The results of our study provide insight into the dynamics of ant-aphid mutualisms in subalpine ecosystems where ants occupy important ecological roles across several trophic levels. Ants act as predators, prey, and decomposers, which is why it is important to expand our understanding of ant ecology and its relationship to other organisms.

There are several possible directions for future research that could expand on the findings of our study. Examining the chemical composition of aphid honeydew could provide insight into ant preferences regarding aphids and other sugar sources. We could also study the association between red aphids and tree health to see if the red aphids have a preference for sick trees. Furthermore, a long-term monitoring study could help us understand how ant-aphid mutualisms respond to changes in weather and seasonal conditions.

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REFERENCES


