ORIGINAL ARTICLE

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Defensive behavior of ants in a mutualistic relationship with aphids

Abstract Mutualistic relationships between ants and aphids are well studied but it is unknown if aphid-attending ants place a greater relative importance on defending aphids from aphid-predators or from competing ant colonies. We tested the hypothesis that aphid-attending ants defend their aphids against aphid-predators more aggressively than against ants from neighboring colonies. We conducted introduction trials by placing an individual non-predatory insect, an aphid-predator, or a foreign conspecific ant on the leaf of a resident ant. We found that ants did not attack non-predatory insects, but did attack competing ants and aphid-predators. When we presented resident ants with both the threats (i.e., predator and competitor) at the same time, residents always attacked potential competitors as opposed to aphid-predators. We suggest this behavior may reduce the likelihood of raids by neighboring colonies. Ants appear to balance both the energetic costs of making an attack and the costs associated with losing aphids to a predator, against the benefits of signaling their defensive ability to rivals and/or preventing rivals from gaining knowledge of a potential food resource.

Keywords Aphids · Ants · Mutualism · *Formica aserva* · Competition · Predation · Ladybird beetle

Introduction

Ants (family Formicidae) are a highly successful taxon that is abundant worldwide. They have achieved their successful radiation largely through social modifications (Wilson

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1963). Ants have evolved distinct division of labor within colonies that allow for organized management of a collective group of related individuals (Weir 1958), and this intra-colony cooperation has enhanced colony survival and species distribution (Wilson 1963). Some ant species are unusual among social animals in that they have also evolved complex inter-specific social relationships. These include species that may enslave ants of neighboring colonies (Hölldobler and Wilson 1990) or species that form mutualistic relationships with other types of insects.

One such mutualistic relationship occurs between honeydew-producing aphids and ants (Buckley 1987; Hölldobler and Wilson 1990). Typically, in this relationship, aphids form dense clusters on deciduous leaves and stems, and ants meet their own carbohydrate requirements by collecting honeydew excreted by the phloem-feeding insects. Aphids benefit from ant attendance through a reduction in predation and parasitism, reduced fungal infection and, in some species, enhanced reproduction (Way 1963; Addicott 1979; Volkl 1992, 1997; Tilles and Wood 1982). Tilles and Wood (1982) showed that aphid colonies disappeared when ants were excluded and that attended colonies were more likely to survive, produce oviparae, and had a higher proportion of wingless adults relative to winged, dispersing alates than unattended colonies. Aphid colonies appear to thrive when attended by ants and much of this success can be attributed to the protection ants afford (Banks and Macauley 1967; Chiverton 1986).

The level of mutualism between aphids and ants depends on various factors, including host density (Addicott 1979; Itioka and Inoue 1996), host plant quality (Breton and Addicott 1992), species-specific differences between the aphid and ant species involved (Addicott 1978), seasonal differences in the honeydew demand of ants (Bristow and Yanity 1999), and differences in the distance between the aphid group and the ant colony (Ryti and Case 1992). It seems likely in all cases of ant–aphid mutualism that, for the aphid farms to result in selective benefits for the individuals in colonies of both species, the ants must successfully defend their aphids from aphid-predators like ladybird beetles (Coleoptera: Coccinellidae; Way 1963).

Way (1963) reported that trees lacking ant defenders were essentially devoid of aphids. Depredation on aphids clearly reduces aphid fitness but it may also have selective implications for the ants if predation causes a reduction in their food supply. However, knowledge concerning ant defensive behavior while guarding aphid colonies is based primarily on anecdotal evidence, and few quantitative studies have examined the nature of the protection offered by the ants (Hölldobler and Wilson 1990).

From a resident ant colony's perspective it may be beneficial to invest in sensory and behavioral adaptations that allow them to defend their aphids and honeydew resource from parasitism by rival colonies of ant farmers that also harvest aphid honeydew. Ant–aphid behavioral experiments by Seibert (1992) revealed that aphid-attending *Formica obscuripes* Forel fervently repelled intruding ants. However, it seems likely that the selective implications of intra-specific parasitism will be less intense than the implications of aphid predation. For example, aphid predation by ladybird beetles results in a permanent loss of resources to the aphid-attending ants, whereas parasitism by one or two rival ants should lead only to a short-term loss in honeydew. On the other hand, if rival ant colonies attack neighboring colonies in large numbers to steal aphids, resource losses for the resident colony may exceed those associated with predation on aphids. It is unknown if aphid-attending ants place a greater relative importance on defending aphids from aphid-predators or from competing ant colonies.

Although competition for mutualists is an influential force in plant–animal interactions (Agrawal and Rutter 1998; Bronstein 1998; Rudgers et al. 2003) its importance in animal–animal associations is largely unexplored. Thus, we sought to determine if aphid-attending ants respond similarly to potential aphid-predators (i.e., ladybird beetles), rival ant farmers, or control insects that pose no threat to the resident ant colony's food supply. We also tested to which of the two potential threats (rival ants or aphid predation) ants respond most intensely when the threats occur simultaneously.

Methods

We studied colonies of aphid-attending ants (*Formica aserva* Forel; Hymenoptera: Formicidae) located in trembling aspen dominated forests (*Populus tremuloides* Michx.), near Deep Lake, Saskatchewan (50° 23.64′N–103° 39.516′W). We conducted the experiments from 24 to 29 August, 2003 using three colonies of *F. aserva*; an ecologically versatile species belonging to a genus known to collect and farm aphids for honeydew (Way 1963; Naumann et al. 1999). We conducted trials with aphids (*Aphthargelia* sp.) and their attending ants on trembling aspen (*P. tremuloides*) and balsam poplar (*Populus balsamifera* L.) trees. Ladybird beetles (*Coccinella septempunctata* Lin.) were collected from aspen within 500 m of the study colonies.

We performed two rounds of experiments. The general protocol for the first round involved excising (physically cutting and removing) a leaf with one resident ant and a group of aphids present, placing the excised leaf on a notebook (22 cm by 27 cm), and then introducing foreign insects to the excised leaf. We excised leaves to control for potential confounding effects of communication with colony mates on the behavior of individual ants. We recorded whether or not the resident aphid-attending ant responded by attacking the novel insect. We defined attacks as interactions involving biting, wrestling, and/or clasping. During trials in which we introduced both a predator and rival ant simultaneously, we defined the attacked individual as that which was attacked for the longest duration. We collected individual ants from ten different colonies and conducted each trial with naïve defending ant and intruder individuals. To identify different ant colonies from which to obtain rival ants, we followed ants from each tree to their nest.

To determine if isolation from the colony affected the aggressive responses of ants we repeated the experiment in August 2004 with the same ant colonies, this time using intact leaves still attached to the trees, as opposed to excised ones.

Predator or competitor trials

We first performed two sets of control trials to test whether resident aphid-attending ants would attack insects of no threat to the resident colony's food supply. The first set of trials involved the introduction of a control ant ($n=10$ excised leaf trials and 10 intact leaf trials). This introduced ant was a member of the same colony as the resident ant selected from another aphid farm on a different plant. We used this specific protocol to ensure that the new ant was in fact of the same foraging caste as the ant under investigation. During a second set of control trials we introduced a muscid fly (Diptera: Muscidae) to represent an intruder that is neither beneficial nor detrimental to the aphids or ants ($n=10$ excised leaf trials and 10 intact leaf trials) (Borror and White 1970).

In our first set of treatment trials we tested the hypothesis that resident ants defend aphids from rival ants. We introduced one conspecific *Formica* ant from a neighboring colony, to a resident ant's excised leaf and recorded whether or not the resident attacked ($n=10$ excised and 10 intact leaf trials). Each introduced ant was from a different foreign colony, while the resident ant–aphid farms were from different trees within one colony. In our second set of treatment trials we tested whether the resident ant would defend aphids from predation. During these trials we introduced one naïve ladybird beetle to a resident ant's leaf and recorded the response ($n=10$ excised and 10 intact leaf trials).

To determine whether ants distinguish between threats and preferentially defend against aphid-predators versus competitors, we conducted sets of trials in which we introduced both a naïve ladybird beetle and foreign ant simultaneously ($n=10$ excised and 10 intact leaf trials). Both introduced insects were placed the same distance from the resident ant directly in front of its field of view.

To test the assumption that ants would actually steal honeydew and adopt new aphids, and not refuse them based on a chemical signature or other markings, we experimentally introduced aphids collected from the ant farms of neighboring ant colonies. We performed trials in which we introduced an aphid collected from a rival colony's farm to an excised leaf with a resident ant and group of aphids, and recorded the response of the resident ant (n=10 excised and 10 intact leaf trials).

Statistical analysis

We recorded all responses as binomial data (e.g., attack or no attack) and used a contingency table analysis to compare attack rates of resident ants to expected attack rates. We could find no basis in the literature for an a priori prediction of how often an ant should attack insects. Therefore, we generated expected values for the analysis based on our observations of resident ant responses to Muscid fly introductions. Muscid flies do not steal honeydew and it is highly unlikely that they are a prey item for these ants so attack rates on muscids provide a good prediction of the probability of an ant attacking any moving insect. To test for heterogeneity we used the Fisher exact test (Zar 1999). Differences between responses were considered significant when $P \leq 0.05$.

Results

In all trials the outcome was unambiguous. Either the ant fought the intruder aggressively (attack), or appeared to ignore it completely (no attack). Resident ants approached all introduced insects immediately but, except for ladybird beetles, only responded (by attacking or not attacking and returning to attending their aphids) after first palpating the introduced insect with their antennae. In all ladybird beetle trials the resident ant responded immediately prior to any palpation.

Predator or competitor trials

Resident ants rarely attacked control insects. Muscid flies elicited attacks in only 20% (2/10) of trials on excised leaves and 10% (1/10) of trials on intact leaves. This attack frequency (i.e., 15%, calculated by pooling results of excised and intact leaf trials) was used to predict expected attack rates for comparison with attack rates during the other trials. The introduction of aphid-attending ants from within the same colony always produced a "no attack" response (P<0.001).

The introduction of an aphid-attending ant from a different colony always resulted in an attack response in both the excised leaf trials (P<0.001) and the intact leaf trials (P<0.001). In all cases, the resident ant approached the introduced ant, palpated it, and then quickly attacked it. Similarly, the response of resident ants to the introduc-

tion of an aphid-predator was to attack. In all trials the resident ant immediately attacked introduced ladybird beetles (P<0.001). Ants may recognize ladybird beetles more quickly than rival ants because they immediately attacked without pausing to palpate them.

When confronted with both the rival ant and the ladybird beetle, simultaneously, the resident ant attacked the intruding ant in 90% of trials (n=20 excised and 20 intact leaf trials). This is significantly more often than expected based on Muscid fly attack rates (P<0.001) and also significantly different from an attack rate of 50%, which would be predicted if ants were equally likely to attack both types of intruders (Sign test, P<0.001). Typically, the resident ant would first pursue and attack the ladybird beetle. However, in all but four cases (two instances on the excised and two on the intact leaf trials) the resident ant quickly abandoned the attack on the ladybird beetle and commenced an attack on the intruding ant. In all of these attacks, the ladybird beetle consumed large numbers of aphids while the resident ant fought with the introduced ant.

Aphid adoption trial

Resident ants responded to introduced aphids collected from the farms of rival colonies, by adopting them into the group they were already tending in 100% of trials (n=10 excised and 10 intact leaf trials). The resident ants carried the new aphids to specific positions on the excised leaf where the aphids immediately began feeding.

Discussion

Competition for food resources between ant colonies has been hypothesized to be important for the evolution of territoriality and zealous defense against food robbing (Yamaguchi 1995) and aggressive behavior also extends to the protection of aphids from natural aphid-predators such as ladybird beetles (Hölldobler and Wilson 1990). Our results are consistent with these findings. When confronted with either a rival ant competitor or an aphid-predator, resident ants invariably responded by attacking the intruder. However, our results also demonstrate that *F. aserva* are not equally aggressive towards all insect intruders that happen upon their aphid farms and apparently make decisions about the risk/cost of different types of intruders. Resident ants were able to identify and avoid attacking insects that posed no threat to their resources. Sister ants were invariably palpated and then ignored and left on the leaf and muscid flies were attacked only rarely. Indeed, some introduced sister ants simply began helping resident ants attend the aphids. Attacking insects that pose no threat would obviously represent an unnecessary energetic cost for resident ants. Our findings suggest that selection has favored mechanisms that allow aphid farmers to identify threats to their food resources and avoid wasting energy by attacking non-threats.

Our results also demonstrate that ants can differentiate between different types of threats to their aphid resources, and make decisions about which threats to address, if they occur simultaneously. Surprisingly, resident ants preferentially defended against rival ants rather than attacks from an aphid-predator. We predicted that residents would respond more aggressively to an aphid-predator because, presumably, predators will cause a more permanent reduction in the food supply than a rival ant making off with honeydew or a few aphids. By choosing to attack the introduced ant rather than the ladybird beetle, resident ants in our study left beetles free to consume large numbers of aphids. One possible explanation for this could be that residents signal their defensive capabilities by attacking rivals. An aggressive attack could prevent a rival ant from returning to its home colony, reporting a weakly defended resource and initiating a raiding party (Hölldobler and Wilson 1990). An attack by the resident may convince a foreign ant that the resource is heavily defended and not worth the effort of a raid (Hölldobler and Wilson 1990). Attacks by residents may also serve as a form of interference competition to prevent the invading ant from sampling the aphids and thereby acquiring knowledge about the resource in the first place (Hölldobler and Wilson 1990). Thus, during the presentation of simultaneous threats, ants appear to balance both the energetic costs of making an attack and the costs associated with losing aphids to a predator, against the benefits of signaling their defensive ability to rivals and/or preventing rivals from gaining knowledge of a potential food resource.

Having said that, it is well known that non-aphid farming ants also attack conspecifics from neighboring colonies. Thus, attacks by residents on intruding ants may not solely reflect a response associated with competition for aphids and honeydew. Other studies have found that the processes structuring, and maintaining ant inter-colony mosaics on the landscape are highly complex and can be motivated by many considerations (Ribas and Schoereder 2002). For example, agonistic interactions between different colonies may reflect competition for other food resources, competition for space, or other stochastic processes (Vepsäläinen and Pisarski 1982; Ribas and Schoereder 2002). It would be extremely difficult to tease apart one potential motivation as the key factor influencing behavior in the colonies of ants we studied (Floren et al. 2001; Ribas and Schoereder 2002). However, it seems likely that the aggressive behavior of ants in our study was strongly influenced by their aphid-farming lifestyle. The farms of each colony we studied were physically large (ca. 10–20 trees and about 100 m^2 in size). Thus, a large proportion of colony members were almost certainly involved in aphid farming, as opposed to other activities. Furthermore, it is well established that species in this genus of ants, and *F. aserva* in particular, often farm aphids so it is likely that considerations associated with ant–aphid mutualism have had a strong influence on the evolution of their aggressive behavior. In any case, even if the aggressive behavior of ants we observed is only partly motivated by costs associated with inter-colony competition for aphids and honeydew, our results still demonstrate that benefits as-

sociated with repelling invading ants, for whatever reason, outweigh the costs of permanently losing a valuable food resource. It is clear that repelling conspecifics from other colonies is a high priority for these ants.

We also found that isolating ants from their colonies by excising leaves had no effect on their aggressive responses. Ants responded identically whether or not they were on intact or excised leaves. This suggests that the decisions made by individual ants during aggressive encounters are not influenced by cues from colony mates. However, we cannot rule out the possibility that proximity to colony mates could influence the decisions of resident ants. Even in our intact leaf trials, all residents were alone on their leaves when other insects were introduced. It would be interesting to repeat these experiments using different sized groups of resident ants on the same leaves. For example, if four resident ants still focused entirely on repelling a single invader while ignoring an aphid-predator it would suggest even more strongly that repelling invading ants provides a very strong fitness advantage. If, however, some ants in the resident group attacked the intruding ant while some attacked the predator, it would indicate that ants not only identify and choose between different types of threats during aggressive encounters but that they can also integrate cues from colony mates into these decisions and perhaps even predict potential outcomes of aggressive interactions.

Our findings demonstrate that ants distinguish between different threats to their aphid resources and make decision about how to respond to these threats. We argue that, by attacking rival ants, residents may reduce the chance that a neighboring colony will initiate a raid. It seems that the fitness benefits of this aggressive behavior, over the long-term, outweigh costs associated with losing aphids to a predator but more work is needed to quantify the selective implications of threat discrimination for aphid-attending ants.

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