Ant behavioral responses to aphid predators in high and low traffic environments

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Abstract

Mutualistic relationships are a critical component of behavioral and community ecology because these relationships help organisms survive, grow, and reproduce. Specifically, in mutualistic relationships, one organism may provide protection from predation for another individual. We sought to determine if the mutualism between ants and aphids is affected by human activities, particularly disturbance due to human foot and vehicular traffic. To determine if ant behavior in response to aphid predators varied between high and low traffic areas, ant species composition, and variation in average temperature, we measured protective and non-protective ant response behaviors on milkweed plants at the University of Michigan's Biological Station. We also collected biological samples of ant species found on each milkweed plant we sampled and took average temperature readings before and after our sampling period. We found that tending behaviors were significantly greater in high traffic areas and ignoring behaviors were significantly higher in high traffic areas when aphid predators were not present. Additionally, ant species composition and average temperature did not have a significant effect on ant behavior. Our results show that ants exhibit protective behaviors in response to aphid predators in general, suggesting that ants benefit from the resources they receive from aphids and mutualistic relationships between these two organisms are strong in this geographical region. Our findings are relevant to ecological scientists interested in researching mutualistic relationships and human influences on the environment, as well as those involved in agriculture and land development industries investigating insects that provide important ecosystem services and pests that cause large amounts of crop losses each year.

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Introduction

Mutualistic relationships between organisms are characterized by interactions that benefit both individuals and play a central role in behavioral ecology. One such relationship is observed between aphids (family *Aphidoidea*), small insects that secrete a sap referred to as honeydew and are viewed as pests in gardening and farming communities, and ants (family *Formicidae*), small terrestrial insects that organize themselves into colonies. In this direct mutualistic relationship, ants "farm" aphids on a variety of plants by herding the aphids, providing them shelter, and protecting them from predation. In return the ants receive food and nutrition by stroking the backs of the aphids, who subsequently excrete honeydew produced by consuming the plant material they live on (Way, 1963). This relationship has been studied extensively and is relevant because both ants and aphids contribute greatly to ecosystems and agriculture in a variety of ways; for example, ants are important soil engineers that make resources available for other organisms (Jouquet, Pascal, et al., 2006) and aphids are pests that contribute to the loss of large quantities of crops every year (George and Gair, 1979). Thus, it is crucial to take this mutualistic relationship into consideration when making decisions on agricultural issues and land development decisions.

In the context of ant-aphid relationships and disturbance by predators, it is important to consider what role human impacts on the environment play in these relationships. Previous studies have shown that disturbance by predators on aphid populations does not necessarily lead to the consumption of these organisms (Nelson and Rosenheim, 2006) and ants place greater priority on attacking neighboring ant colonies when they approach their aphid farms opposed to attacking aphid predators (Phillips and Willis, 2005). Additionally, humans are increasingly affecting natural habitats by developing land that is home to organisms providing important ecosystem services (Metzger, 2006). However, few studies have directly addressed the role that human impacts on aphid-ant relationships play in ant behavioral responses to predators. This study aims to fill this knowledge gap by examining how ants farming aphids respond to aphid predators in areas with high amounts of human foot and vehicular traffic compared to areas with low human-related traffic.

Additionally, this study examines the role that different ant species might play in ant behavioral responses to predators. Past studies have shown that certain species of ants respond differently to aphid predators; for example, one species only guarded aphids in response to adult ladybird beetles and ignored all other predators, while another ant species attacked all predators in the adult and larval forms (Novgorodova, 2005). However, few studies have examined the differences in ant species and behaviors between high and low traffic areas. This study aims to fill this gap by determining if variation in ant species exists between high and low traffic sites, and if this species variation plays a role in the observation of more protective or non-protective ant behaviors in the presence of aphid predators.

We examine the protective and non-protective behaviors of ants in response to aphid predators on milkweed plants (family *Asclepiadoideae*) in northern Michigan with a focus on high and low traffic areas, ant species composition, and average soil temperature at experimental sites. Although past studies have shown that ants tend to exhibit protective behavior in the presence of aphid predators (Bryson, 2000) and certain species of ants responds differently to aphid predators (Novgorodova, 2005), we aim to expand upon these results by examining ant behavior in response to aphid predators in high and low human foot and vehicular traffic areas. More specifically, we address three questions: (i) Will ants exhibit protective behaviors in response to aphid predators more often in high or low traffic areas? We hypothesized that ants would exhibit more protective behaviors in high traffic areas because these ants are more accustomed to disturbance by humans and thus are conditioned to respond with protective behaviors. (ii) Will ant species composition vary depending on distance from the road, and if so, might this contribute to a difference in observed ant behaviors? We predicted that ant species richness would be relatively similar at low and high traffic sites due to the small area of our sampling sites, having no effect on ant behaviors. (iii) Will average temperature at high and low traffic sites have an effect on ant behavior? We hypothesized that higher average soil temperatures would lead to greater amounts of protective ant behaviors because ants are more active and aggressive in warmer temperatures. We investigated these questions by observing protective and non-protective ant behaviors in response to aphid predators on milkweed plants. We analyzed the results in the context of behavioral and species variation.

Methods

Study sites and environmental data

At the University of Michigan Biological Station in Pellston, Michigan, USA, we observed ant behavior in response to aphid predators and collected average temperature readings on July 29 and August 3, 2015. Specifically, we sampled two sites in the Biological Station's UV field, an open area containing many shorter herbaceous plants and tall grasses. We selected the UV field for our study because it has two areas with high densities of milkweed found at varying distances away from the two-track road that transects the field. Thus, we were able to compare ant behavior in the presence of aphids on milkweed plants close to the road to lower traffic areas. Additionally, at both sampling sites, we took soil temperature (°C) readings before and after our observational sampling periods on both days of our experiment at regions 0-10 m and 10-20 m away from the road. We averaged the before and after temperatures at each site to determine the average temperature during our sampling.

Ant Behavior

To measure ant behavior in the presence of aphid predators, we selected two 20 m x 15 m areas of the UV field that had plentiful amounts of milkweed plants containing aphid and ant populations. In these two areas, designated as area 1 and area 2, we used a meter tape to measure 20 meters away from the road. We divided the areas into two sections to compare high human foot and vehicle traffic (0-10 m, site 1) and low traffic (10-20 m, site 2) environments. At both areas, we observed ant behavior on five milkweed plants, designating one plant as a control, at which we observed ant behavior without the influence of a predator. At the other four plants, we used a paintbrush to mimic an aphid predator by placing the brush on either a leaf or a flower that had high densities of aphids and ants (figure 1). We moved the brush around the aphid populations for three minutes and recorded each ant behavior observed in that time period. We observed ant behavior on a total of twenty plants and conducted our experiment two times for a total of forty trials.

Ant behaviors recorded were split into two categories: protective and non-protective (Bryson, 2000). Protective behaviors included “tending” to the aphids by encircling the colony or herding the insects, “attacking” the predator-like paintbrush by engaging in physical contact with the brush, and “assisting” the ant and aphid populations facing predation by coming onto the leaf with the predator. Non-protective behaviors include “leaving” the plant and avoiding the predator, as well as “ignoring” the predator and aphids by exhibiting no responsive behaviors.

Finally, once the three-minute observational period was complete, we randomly selected two ants off of each plant and placed them in a vial of ethanol to preserve the samples (figure 2). Each area’s 0-10 m and 10-20 m site biological ant samples were collected in the same jar to serve as a random sampling of the ant species present in each area. We brought ant samples back to the lab for species identification.

Analysis of ant behavior and temperature data

To determine if there was a significant relationship between ant protective versus non-protective behaviors in low or high traffic areas or in the presence of predators, we ran a two-way ANOVA analysis on five conditions. Additionally, to evaluate if differences in ant species composition existed between high and low traffic areas, we ran an independent samples t-test. Finally, we ran an independent samples t-test to determine if average temperature during our sampling periods differed between sites, potentially having an effect on ant behavior.

Figure 1 – Decoy aphid predator near aphid colony

Figure 2 – Collection of ant species samples

Results

Ant behavior

To analyze ant behavior in the presence of aphid predators and the distance from the road, we ran a two way ANOVA analysis on five conditions. We found that the protective behaviors of assistance and attacking were significantly higher in the presence of aphid predators (p=0.003 and p=0.00, respectively). Additionally, tending was significantly higher closer to the road (p=0.029). Finally, when considering both the presence of aphid predators and distance away from the road, we found that ignoring was the only significant behavior observed (p=0.016). Ignoring behaviors were observed closer to the road and when aphid predators were not present. For a graph of the averages of all observed behaviors, see Appendix, figure A. Thus, we determined that ants did not exhibit significantly more protective behaviors in high traffic areas.

Ant species composition

To determine if ant species composition varied significantly between high and low traffic sites, we ran an independent samples t-test. We found that ant species composition, including ants from the genuses *Formica*, *Lasius*, and *Camponotus*, was not significantly different between sites (Appendix, figure B). Therefore, we concluded that species variation does not have an effect on ant behavior.

Temperature

By running an independent samples t-test on the average temperature (°C) data for each site, we found no significant difference in average temperature between sites 1 and 2 (p=0.853). Therefore, we concluded that average temperature at each site on the days of sampling did not have an effect on observed ant behaviors.

Discussion

As humans have increasingly negative impacts on the environment and contribute to environmental degradation and habitat fragmentation, it is important to consider how human disturbance affects mutualistic relationships. Mutualistic interactions are crucial to maintaining natural communities (Christian, 2001); thus, the breakdown of these relationships due to ecosystem disruption by humans has the potential to effect evolutionary processes and lead to loss in biodiversity, given that organisms in mutualistic relationships are often bound to a common fate (Kiers et al., 2010). One mutualistic relationship that is relevant in agricultural spheres involves ants and aphids. Ants are important ecosystem engineers that contribute to soil functionality and the alteration of physical and chemical processes; additionally, ants are highly responsive to anthropogenic effects on the environment (Folgarait, 1998). Therefore, although aphids are parasites that contribute to large amounts of crop losses every year (George and Gair, 1979), they support ant populations by providing them with food and nutrients. In return, aphids receive shelter and protection from predation from the ants. Understanding how this mutualism is affected by human activity is crucial to determining potential changes in ant and aphid biodiversity and the impacts this mutualism has on farming communities. Further empirical study on this mutualism and its responses to human influence is needed to fully understand how ants respond to aphid predators in high human traffic environments, and how ant species composition and average temperatures affect ant/aphid mutualisms.

Contrary to our first hypothesis, we did not see significantly higher amounts of ant protective behavior in response to aphid predators in higher traffic areas. However, we observed significant amounts of attacking ($p=0.00$) and assisting ($p=0.003$) in the presence of aphid predators in general. We determined there were significant amounts of tending ($p=0.029$) when not considering the presence of aphid predators. Additionally, we found that ignoring was significantly higher closer to the road in the absence of aphid predators ($p=0.016$). We also determined that average temperature at the study sites had no significant effect on ant behavior. Lastly, we found that ant species composition was relatively even between high and low traffic sites, having no effect on ant behavior.

First, when considering why our hypothesis that ants would exhibit more protective behaviors in response to aphid predators in high traffic environments was rejected, we must consider the roles that sampling methods and the location and characteristics of the study site might have played in our findings. Given that we observed significantly higher amounts of attacking and assisting in the presence of aphid predators, a finding also supported in Bryson (2000), we determine that ants do respond to aphid predators with protective behaviors in general. Additionally, tending was significantly higher nearer closer to the road when not considering the presence of aphid predators. Ants in higher traffic areas may be conditioned to protecting aphids due to frequent disturbance by human traffic; however, we cannot make this conclusion without showing that there is a significant correlation between the distance from road and the type of behavior displayed. The only significant behavior observed when considering both variables was ignoring, which was higher closer to the road and in the absence of aphid predators. It is possible that our methods of tallying ant behaviors on control plants were inconsistent; technically, our definition of ignoring was the observation of no responsive behaviors to aphid predators. However, when tallying behaviors on control plants without the presence of predators, the observation of ants moving about the plant without interacting with aphids was often tallied as an ignoring behavior. Thus, inconsistencies in sampling of ant behaviors may have lead to the observation that ants in high traffic areas ignore more often when predators are absent. Additionally, the characteristics of our sample sites may have been too similar to accurately make comparisons between high and low traffic sites. For example, although site 1 was closer to the road than site 2, both sites are located in a relatively remote field that receives most human traffic in the form of bicyclists and runners. Therefore, future studies should compare sites that have almost no human traffic in any form with those having much heavier human traffic, including frequent disturbance by automobiles.

Our sampling methods for ant species composition may have also played a role in the observation of no significant difference between ant species and behaviors. Although our results were in agreement with our hypothesis that ant species composition would not have an effect on ant behavior, this observation may have been due to sampling techniques rather than the small area of our sampling locations. Instead of measuring the behavior of each ant species, we took a sample of two random ants from each plant to determine the average makeup of the ant species present in high and low traffic areas. Thus, even if ant species composition was not relatively similar across all sites as we observed, we would not have been able to accurately determine the behaviors exhibited by each species using our sampling methods. However, Novgorodova (2005) determined that certain species of ants respond differently to aphid predators by marking individual ants with paint to distinguish between species and observing ant behavior on aphid colonies. Future studies aiming to compare ant behavior between species should consult this literature and methodology.

When evaluating why average temperature was consistent among sites and therefore did not lead significantly affect ant behavior, we must consider the role that the duration of our experiment played in this observation. We collected temperature data over the course of an hour on two days; however, this data is not necessarily representative of the longer-term temperature trends and the variation in climate that exists in the area. Although past studies have shown that higher temperatures lead to the observation of less aggressive ant behaviors (Barton and Ives, 2014), this may not have been observed in our experiment because of the homogeneity of the climate in the area and the temperatures on sampling days. Therefore, future studies aiming to compare the effect of temperature on ant behaviors may want to compare two different climates or collect temperature readings for a longer period of time.

The main takeaway point from our experiment is the importance of consistent experimental design. Although we did find significant amounts of protective behaviors exhibited in ant populations in the presence of aphid predators, our study location and sampling methods may have hindered the ability to accurately observe differences in ant behaviors between high and low traffic areas. Further empirical study is needed to determine human influences on the mutualism between ants and aphids.

Appendix

Figure A – Totals of average ant behaviors recorded with aphid predators present (attack) and absent (control)

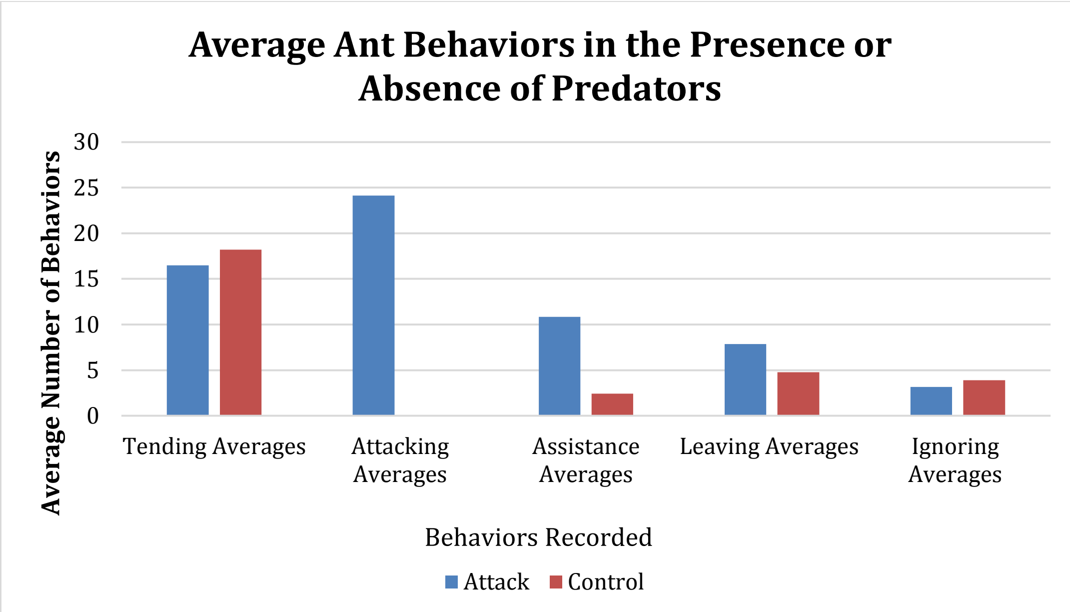
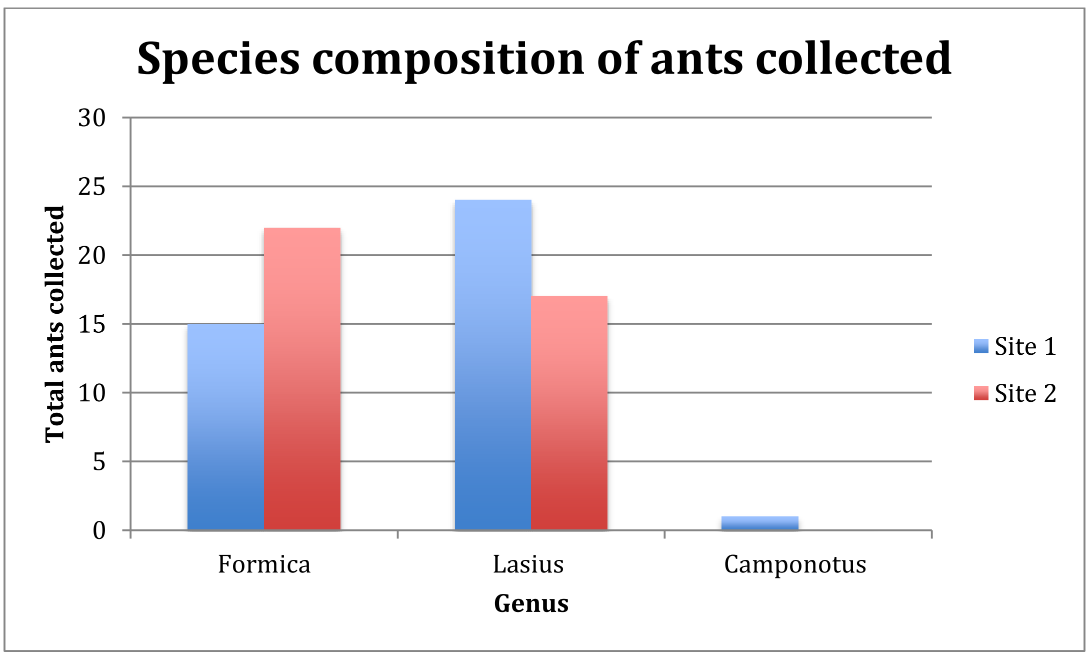


Figure B – Species composition of ants is relatively similar for sites 1 and 2



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