Measuring Ant-Aphid Mutualism with Human Disturbances

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Abstract

Ants and aphids have long been known to display a mutualistic relationship. However, not as much is known about how ant behavior changes with habituation of the ants to disturbances. We wanted to know if ants are used to being disturbed, how would they react differently to the predator or a disturbance, if at all. To test this we used the UV field at the University of Michigan Biological Station that has a plethora of milkweed, ants, and aphids. Our disturbed area was the first ten meters on the side of a road and the undisturbed area was the 10 meters following. We watched for five behaviors in the ants while acting as a pseudo-predator for three minutes and tallied these behaviors. We found that ants are more likely to ignore the predator the closer to the disturbed area that the ants are located. However, the ant-aphid mutualistic relationship has a broader applicability when considering monarch butterflies. Although ants protect the aphids, they also protect the plant. With the Midwest providing a home for so many monarchs, adding ants and aphids to milkweed plants across the Midwest could also protect the monarch and help restore their numbers to historic levels.

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Ants and aphids have long been known to display a mutualistic relationship. However, not as much is known about how ant behavior changes with habituation of the ants to disturbances. We wanted to know if ants are used to being disturbed, how would they react differently to the predator or a disturbance, if at all. To test this we used the UV field at the University of Michigan Biological Station that has a plethora of milkweed, ants, and aphids. Our disturbed area was the first ten meters on the side of a road and the undisturbed area was the 10 meters following. We watched for five behaviors in the ants while acting as a pseudo-predator for three minutes and tallied these behaviors. We found that ants are more likely to ignore the predator the closer to the disturbed area that the ants are located. However, the ant-aphid mutualistic relationship has a broader applicability when considering monarch butterflies. Although ants protect the aphids, they also protect the plant. With the Midwest providing a home for so many monarchs, adding ants and aphids to milkweed plants across the Midwest could also protect the monarch and help restore their numbers to historic levels.

Introduction

Any two organisms in the world can interact in a plethora of ways. These relationships can benefit, one, neither, or both of the organisms. Ants and aphids, like many other living organisms, interact on a daily basis. However, their relationship can be much more mutualistic than those otherwise experienced in nature (Addicott, 1978). Mutualism is defined as “an interaction between species that is beneficial to both” (Boucher et al., 1982). Generally, it is agreed that ants and aphids demonstrate this relationship. The aphids gain protection from the ants’ presence and the ants have a stable food source from the honeydew that the aphids produce (Bryson, 2000). Without the ants, the aphids are more vulnerable to predation and without aphids, ants are much more susceptible to changing environments because of the lack of a consistent food source (Piovia-Scott, 2010).

Aphid-ant relationships are subject to the most stress when predators are involved. Ants are able to sense a pheromone produced by aphids as an alarm signal (Vandermoten et at., 2012). The release of this signal causes the ants that protect them to become much more aggressive towards the predator (Nault et al., 1976). While this relationship varies between aphid species, predation is still high for those moving and non-moving aphids and effects the ants’ ability to defend (Nelson & Rosenheim, 2005).

However, the ant behavior towards the predator when looking at habituation and distance has rarely been studied before. In order to test if ants can become habituated to the release of this pheromone and start to ignore the signal produced by the aphids, we wanted to test the difference in response in high and low traffic areas.

We hypothesize that due to higher traffic, the ants closer to the road will become more habituated to disturbances and therefore less aggressive towards the predator. We also hypothesize that with higher temperatures, there is an increase in protective behaviors because of the high stress environment forcing the ants to rely more heavily on their food source, as well as ants also have a higher metabolism rate in hotter temperatures, which might make them consume more honeydew (Youngsteadt et al., 2014). Compared to the control plants, we expect to see more aggressive behavior on the plants we are attacking and more tending on the plants we are simply watching. Lastly, we hypothesize that species will have no effect on behavior because of the limited number of ant species in Northern Michigan.

Methods

To measure ant-aphid interactions and the effect of high and low traffic on these interactions, we started by dividing our area, the UV field at the University of Michigan Biological Station, into two groups: high traffic, 0 – 10 meters from the road, and low traffic, 11-20 meters from the road. In each section, we found 10 milkweeds total to study, nine to test and one to watch as a control. For our pseudo-predator we used the back end of a paintbrush and disturbed the leaf around the aphids with the intent of not actually harming or touching the aphids.

Working in groups of two, we watched the ants' response to the predator on one leaf for three minutes with one person calling out the behaviors and the other recording them. The ants could display five different behaviors: leaving, assisting, attacking, tending, and ignoring. Leaving happened whenever an ant left the one leaf we were "attacking". Assisting happened when an ant came onto the leaf that was not previous there. Attacking happened when an ant came onto the paintbrush. Tending happened when an ant nurtured the aphids. Ignoring was any other behavior, typically found when the ants simply walked around the leaf instead of exhibiting one of the other behaviors outlined above. These behaviors can be further categorized into protective – attacking and assistance – and non-protective – leaving, ignoring, and tending.

After recording the behavior on each plant, we collected two random ants from the leaf in order to determine if ant species can account for differences in behaviors. The ants were placed in a vial filled with ethanol based off of which area we were in. The ants from 0-10m were placed in one vial and 10-20m in a second vial. This was repeated at the second site with two more vials being used for the two distances. We also took the temperature before and after the experiment. We repeated this process over two days to obtain enough data. We categorized the ants collected based off of genus after returning to the classroom.

After tallying the behaviors, we analyzed the results using SPSS to determine if any major differences happen between high and low traffic areas, as well as between control and "attack" plants. We used a two way ANOVA with five conditions to cross reference the five different behaviors with our other two categories, attacking versus controls and closer to the road and further. We also ran two Independent Samples T-Test. The first test we ran we compared species composition and distance of the road. The second T-Test we ran was comparing temperature and distance from the road.

Results

After running the tests outlined above we found three main results from our 2-way ANOVA. When comparing the results from plants we attacked to our control plants, we found that both attacking and assistance had a significant difference ($p = 0.000$; $p = 0.003$). When looking at the two distances, 0-10m and 10-20m, the only significant difference in the five behaviors is present in tending, with more tending happening closer to the road ($p = 0.029$). When comparing both attacked versus control plants and the distance from the road, ignoring was much more prevalent closer to the road (Figure 1) and when not being attacked (Figure 2; $p = 0.016$).

For our Independent Samples T-Test comparing the species collected and the distance from the road, we found no significant relationship between the two. In the second Independent Samples T-Test comparing temperature at the two sites and distance from the road, we also found no significant relationship.

Discussion

In this study we hypothesized that ants would attack more on “attack” plants than control plants, when disregarding distance from the road. We found that attacking and assisting were significantly different between the two ($p = 0.00$; $p = 0.003$). It is important to note that attacking had such a low p value because it was impossible for an ant to “attack” on a control plant because of the absence of a predator. These data demonstrate that our experiment worked because the ants reacted significantly differently in two behaviors of the five when being attacked or not attacked. These data also show that a mutualistic relationship does exist between the ants and aphids because of the help that the ants provide the aphids with their defenses.

More specifically, we hypothesized that ants would become more habituated to disturbances closer to the road and therefore react less to the “attack” we created. We found that ignoring and tending are more prevalent closer to the road ($p = 0.016$; $p = 0.029$), matching our hypothesis. This could perhaps be because the ants become accustomed to the chemical signal that the aphids release, making them ignore our presence more frequently the closer to the road they were located (Vandermoten et at., 2012). However, tending was only significant when looking at distance from the road, not with attacking versus not attacking. Ignoring was significant when looking at both variables, and occurred more frequently closer to the road when no attacker was present. Ignoring could have been more abundant because of the ambiguous nature of our definition. Ignoring could have referenced either the aphids, predator, or any behavior that was not the other five and therefore skewed the data some.

We also hypothesized that temperature would effect the metabolism of the ants forcing them to rely more heavily on the aphids, and therefore make them protect the aphids more the hotter the temperature. However, contrary to our prediction, we found no significant relationship between temperature and distance from the road. Nevertheless, it is common for ants to defend aphids more aggressively in hotter temperatures if they are used to cooler ones (Barton & Ives, 2014). We also hypothesized that ant species would not effect their behavior. Based off our results, we cannot conclude anything from the data and can therefore neither confirm nor deny our hypothesis.

However, possible errors could have occurred throughout the experiment, causing the data to skew. To begin, recording bias occurred throughout since all the data for the behaviors being collected was subject to the individual watching the ants. A more significant error could have resulted from the actual location that we selected to do our study. The “road” we used for the high traffic area, is not frequented as often as other main roads throughout the University of Michigan Biological Station. Although there would be more disturbance at the road than 20 meters off of it, the disturbances could be far and few between. With no clarity for how often the UV road at UMBS is visited, we are not sure how this affected the data.

Taking into account the possible caveats, we can still say that ants react differently the closer they are to the disturbance. The closer they are to the road, the more they ignore the “attacker”, we suggest from the habituation of attackers not affecting the aphids. The ants are also much more likely to attack and assist when under attack, suggesting that a mutualistic relationship does occur and our experiment was effective.

Interestingly, however, ants provide protection for not only the aphids, but also for the plant (Zhang, 2013). With dwindling populations of the protected monarch butterflies (Brower, 2011), adding aphids to milkweed plants to sustain the plant’s health through ant protection, and hopefully the monarchs, could prove fruitful. The decline in milkweed in the US Midwest, where many monarchs come from, could be partially at fault in the monarch declining populations (Pleasants & Oberhauser, 2012). Using the aphid-ant-plant protection mechanism and replanting milkweed in the Midwest it is possible to achieve more bountiful populations of the protected monarch butterfly.

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Appendix

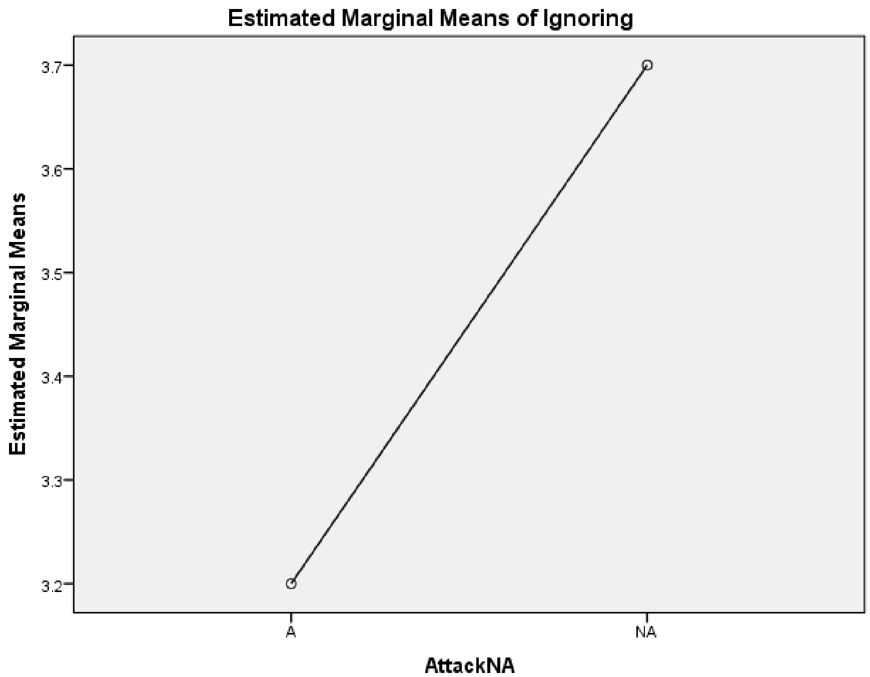

Figure 1: When looking only at our variable attacking versus not attacking, ignoring is higher when not attacking (control) compared to attacking

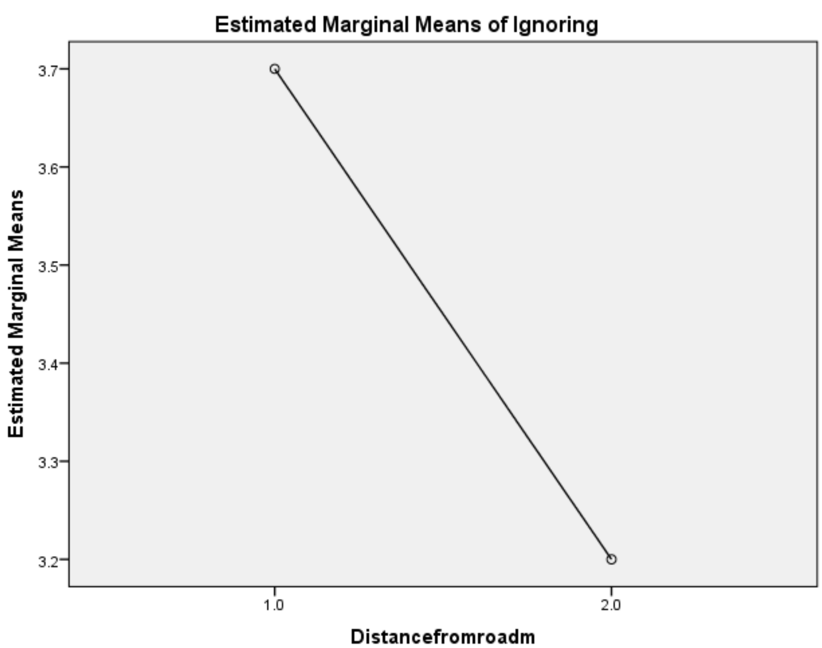

Figure 2: When looking only at our variable distance from the road (m), ignoring is more prevalent the closer to the road the ant is located (1.0 = 0 – 10m from road; 2.0 = 11-20m from road)