Ant-Aphid Mutualistic Systems and Human Disturbance

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EEB 381 General Ecology

8/17/2015

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Abstract: Mutualism is an ideal form of coexistence as both species involved benefit from the behavior of the other. Ants and aphids exhibit a clear mutualistic relationship which has been studied for over 100 years. Our purpose was to study this interaction between ants and aphids and the possible effect of human disturbance on the facilitative relationship. We sampled forty ant/aphid systems on milkweed, twenty within 0-10 meters from the road, and twenty within 10-20 meters from the road. We performed three minute trials, introducing a paintbrush as a mock-predator to the ant's aphid colonies and tallying five behavioral responses: two protective, two non-protective, and one neutral. We found a significant increase in protective behaviors in the presence of a predator, supporting the mutualistic relationship. We did not find any significant difference in protective behavior based on distance from the road, which could be because our distances were too similar. However, we recorded more tending behavior closer to the road that may be due to disturbance causing an increase in plant phloem production that increases herbivorous behavior of aphids, resulting in more honeydew production that would increase ant tending behavior. When there was no predator and the milkweed was closer to the road there was an increase in ignoring behaviors. This could be caused by ants near the road traffic habituating to disturbance. We found only a slightly higher richness of ant species closer to the road, a difference that was insignificant as there was only one ant of a different species found in the sites within 0-10 meters from the road. We found no significant difference between soil temperature close to the road and far from the road, meaning we could make no conclusion about the effect soil temperature has on ant behavior. Ants and aphids are a classic example of a mutualistic relationship. Studies show that mutualistic relationships are crucial for the preservation of natural communities, therefore it is important to study how a very prevalent mutualistic interaction is affected by human disturbance.

INTRODUCTION

Ecology involves the interactions between organisms themselves in the form of competition, predation, or mutualism. Whereas competition and predation involve negative effects for the organisms involved, mutualism is an interaction that benefits both individuals. Ants and aphids are an example of this positive relationship; while aphids provide nutrients for ants, ants provide protection for the aphids.

A mutualistic interaction can be defined as a relationship in which the individuals involved provide benefits in shelter, dispersal, energy, protection, or reproduction to the other (Post *et al.*, 1985). Ants carry juvenile aphids to milkweed for shelter, hide them from plain site, and provide them with protection (Bawaba, 2005). Aphids extract nutrients from the milkweed plant and excrete sugar-rich "honeydew" that ants consume (Bawaba, 2005). Although this relationship is not necessarily an obligate mutualism, it is possible that ants and aphids actively seek out environments where the other exists (Fischer *et al*., 2015).

For 100 years, scientists have studied this mutualistic interaction between ants and aphids. Since 100 years ago, the human population has increased exponentially. Therefore, it is important to study the effect human disturbance might have on ants and aphids. Studies have shown that plants that have been disturbed often produce a higher volume of nutrient-rich phloem, therefore milkweed disturbance could allow the aphids to create more honeydew (Piova-Scott, 2010). However, the milkweed would then suffer more herbivorous attack by the aphids.

We hypothesize that ants will exhibit a significantly larger amount of protective rather than non-protective behaviors when a predator is introduced to the aphid colonies. We also hypothesize that ant colonies in the presence of a predator that are found closer to the road, therefore experiencing more "disturbance", will exhibit a higher amount of protective behaviors. Finally, we hypothesize that ants will be more protective of the aphids when temperatures are higher because the temperature could cause an increase in metabolism or agitation. During our study we will test the effect of disturbance on ant behavior on milkweed containing ant tended aphids.

METHODS

We studied ant responses to invasion of their aphid colonies on July 29th and August 3rd. We studied this mutualistic relationship in forty milkweed plants from two sites along the road in the UV field of the University of Michigan Biological Station, Cheboygan County, Michigan (45°33'N; 84°40'W, T37N, R3W). We selected twenty plants from each site, all containing colonies of active mutualistic ant and aphid colonies and were located within the desired distances from the road to measure traffic effects on ant behavior.

At both sites we extended a meter tape roll from the edge of the road to twenty meters from the road. We then selected 10 milkweed plants that contained ants and aphids within 0-10 meters, and 10 plants within 10-20 meters. We recorded soil temperature for each section on each day. Using tweezers, we collected two ant samples from each milkweed plant on both days, totaling 80 ants, and placed them in small vials of ethanol to preserve them for later identification.

At each plant we performed a three-minute behavior analysis of the ants when a mock-predator of aphids, a paintbrush, was introduced near the aphid clusters. We selected one leaf containing an aphid colony and stroked the paintbrush continuously around the cluster of aphids throughout the duration of the three minutes. We tallied five different ant behavioral responses starting once we introduced the paintbrush. These behaviors were categorized into two protective ant responses (attacking the paintbrush and providing assistance by coming on to the leaf where the invasion is occurring), two non-protective responses (ignoring the attack and leaving the plant), and one neutral response (tending the aphids). We performed these tests once on each of the forty milkweed plants over the duration of the two days.

We ran a two-way ANOVA on five conditions to test for a significant relationship among any of the ant behaviors and presence of a predator or distance from the road. We ran an independent samples t-test to analyze the relationship between ant species and distance from the road. Finally, we ran a second independent samples t-test to determine if there was a significant relationship between soil temperature and distance from the road.

RESULTS

Presence of a Predator

We found there was a significant difference in the level of ant attacking and assistance behavior between the presence of a predator and the absence of a predator (Fig.3, Fig.4). The amount of observed attacking behavior ($p=0.00$) and assistance behavior ($p=0.03$) increased significantly when we introduced a predator to the milkweed (Fig. 1).

Distance of Milkweed from the Road

The only behavior that displayed a significant difference between the sites near to the road and far from the road was tending (Fig.2). We found a significantly higher amount of ant tending behavior closer to the road ($p=0.016$) (Fig.1).

Presence of a Predator and Distance of Milkweed from the Road

The only behavior we found to be significantly different between the presence versus absence of a predator and sites closer versus farther from the road was ignoring. There was a significantly higher amount of ignoring behavior when there was an absence of a predator and the milkweed was closer (within 0-10 m) to the road (p=0.016) (Fig.1).

Ant Species

We found no significant difference in ant species distribution between sites within 0 to 10 meters from the road and sites 10 to 20 meters from the road. We found a significantly higher diversity of ant species closer to the road because the genus camponotus was only found in sites closer to the road (p=0.024).

Soil Temperature

We found no significant relationship between temperature and distance from the road. The sites closer to the road and farther from the road did not have a significant variation in temperature.

DISCUSSION

In this research we analyzed the behavior of ants with aphid colonies and the effect that high traffic areas might have on ant behavior on milkweeds that contain ant/aphid mutualistic systems. We hypothesized that ants would display a significantly higher amount of protective behaviors when we introduced a paintbrush to a leaf of the milkweed plant containing colonies of their aphids. We hypothesized that ants on milkweed closer to the road, experiencing higher traffic, will exhibit a higher amount of protective behaviors. We also hypothesized that higher temperatures would induce a higher amount of ant protective behaviors as a higher temperature could increase metabolism or unrest.

We found a significant relationship between the presence of a predator, meaning the introduction of the paintbrush, and an increase in the amount of ant protective behaviors recorded. This supports our hypothesis that ants would protect the aphids if a predator disturbed them. This also makes evident the mutualistic relationship between ants and aphids as our data supports that ants exhibit more protective behavior when a predator is near the aphid colonies.

We did not find a significant difference in protective versus non-protective behaviors in sites closer to the road and sites farther from the road. We hypothesized that the sites closer to the road, with more disturbance, would have higher protective ant behavior, which was not observed. This could be because the distances were too similar. It is possible that at a farther distance, such as on milkweed 50 meters from a road, there would be a difference in ant behavior from ants on milkweed within 10 meters from the road. We could not find milkweed farther than 20 meters away from the road so we were unable to test at farther distances. We did observe more tending, a neutral behavior, closer to the road which could be due to disturbance increasing phloem production of milkweed caused by nearness of road traffic; herbivorous behavior of the aphids would increase, therefore increasing honeydew output for the ants (Piova-Scott, 2010). This increase in honeydew production would supply more nutrients for ants who would then increase tending behavior.

We recorded more ignoring ant behaviors on the milkweeds closer to the road and when there was no predator present. More ignoring closer to the road could be a result of habituation, or the ants becoming accustomed to disturbance, inducing ignoring behavior. Tending and protecting the aphids could be considered costly for the ants in terms of energy (Bryson, 2000). This may be why we recorded more ignoring when a predator was not present. The ignoring behavior is also quite ambiguous, meaning the ants' motives are unclear, so it is difficult to draw conclusions from the display of this behavior.

We found a significant difference in the distribution of ant species between the closer sites and the farther sites; more specifically we only found camponotus in sites closer to the road, where more disturbances occur. However, other studies with a greater sample size and longer experiment duration have shown that sites that experience more traffic and disturbances have fewer ant species (Graham *et al.*, 2004). All of our site locations were within the same field and in close proximity; therefore, finding a high ant species richness to compare different ant species' behavior was difficult.

Soil temperature did not vary significantly depending on the distance of the milkweed site from the road, so we are unable to make any conclusions on the effect that temperature might have on ant protective behaviors. However, other studies have shown that increased temperature does have an effect on ant protective behavior of aphids. High temperatures can decrease the abundance and aggressiveness of ants in ant/aphid systems (Barton *et al.*, 2014).

Appendix

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Tending	611.154[a]	3	203.718	1.931	.142
	Attacking	5593.729[b]	3	1864.576	8.522	.000
	Assistance	572.367[c]	3	190.789	3.904	.016
	Leaving	114.038[d]	3	38.013	1.556	.217
	Ignoring	73.975[e]	3	24.658	2.733	.058
Intercept	Tending	7965.306	1	7965.306	75.516	.000
	Attacking	3945.735	1	3945.735	18.034	.000
	Assistance	1183.713	1	1183.713	24.219	.000
	Leaving	1101.918	1	1101.918	45.092	.000
	Ignoring	328.817	1	328.817	36.445	.000
AttackNA	Tending	9.773	1	9.773	.093	.763
	Attacking	3945.735	1	3945.735	18.034	.000
	Assistance	498.015	1	498.015	10.189	.003
	Leaving	63.460	1	63.460	2.597	.116
	Ignoring	1.727	1	1.727	.191	.664
Distancefromroadm	Tending	544.248	1	544.248	5.160	.029
	Attacking	341.850	1	341.850	1.562	.219
	Assistance	52.547	1	52.547	1.075	.307
	Leaving	7.345	1	7.345	.301	.587
	Ignoring	1.727	1	1.727	.191	.664
AttackNA * Distancefromroadm	Tending	53.104	1	53.104	.503	.483
	Attacking	341.850	1	341.850	1.562	.219
	Assistance	.173	1	.173	.004	.953
	Leaving	14.148	1	14.148	.579	.452
	Ignoring	58.083	1	58.083	6.438	.016
Error	Tending	3797.221	36	105.478		
	Attacking	7876.671	36	218.796		
	Assistance	1759.533	36	48.876		
	Leaving	879.737	36	24.437		
	Ignoring	324.800	36	9.022		
Total	Tending	15799.000	40			
	Attacking	27458.000	40			
	Assistance	5536.000	40			
	Leaving	3053.000	40			
	Ignoring	841.000	40			
Corrected Total	Tending	4408.375	39			
	Attacking	13470.400	39			
	Assistance	2331.900	39			
	Leaving	993.775	39			
	Ignoring	398.775	39			

a. R Squared = .139 (Adjusted R Squared = .067)

b. R Squared = .415 (Adjusted R Squared = .367)

c. R Squared = .245 (Adjusted R Squared = .183)

d. R Squared = .115 (Adjusted R Squared = .041)

e. R Squared = .186 (Adjusted R Squared = .118)

Figure 1: A two-way ANOVA on five conditions of ant behaviors in relationship to presence and absence of predators or distance from the road, within 0-10 m or 10-20 m.

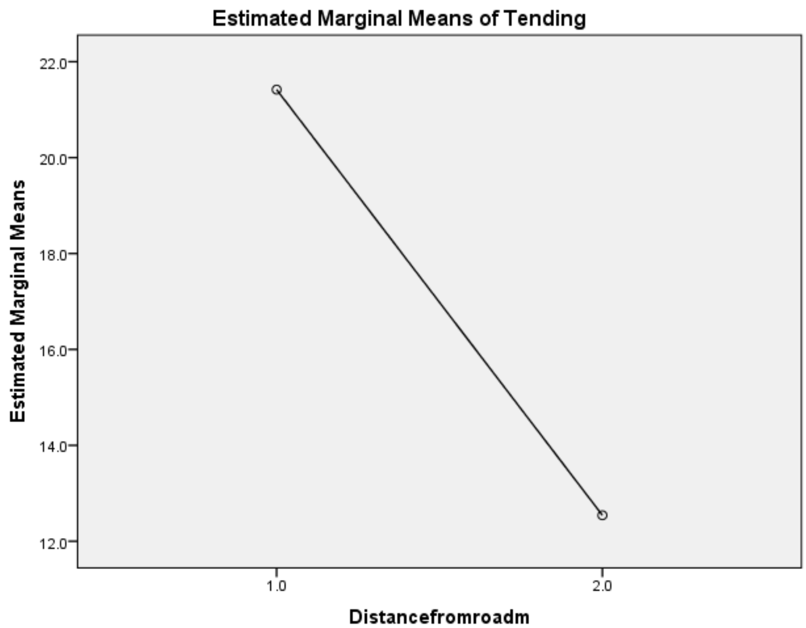

Figure 2: A graph showing the trend of the estimated marginal mean of tending behaviors in relationship to the distance from the road, 1.0 being closer (within 0-10 m) and 2.0 meaning farther (within 10-20 m).

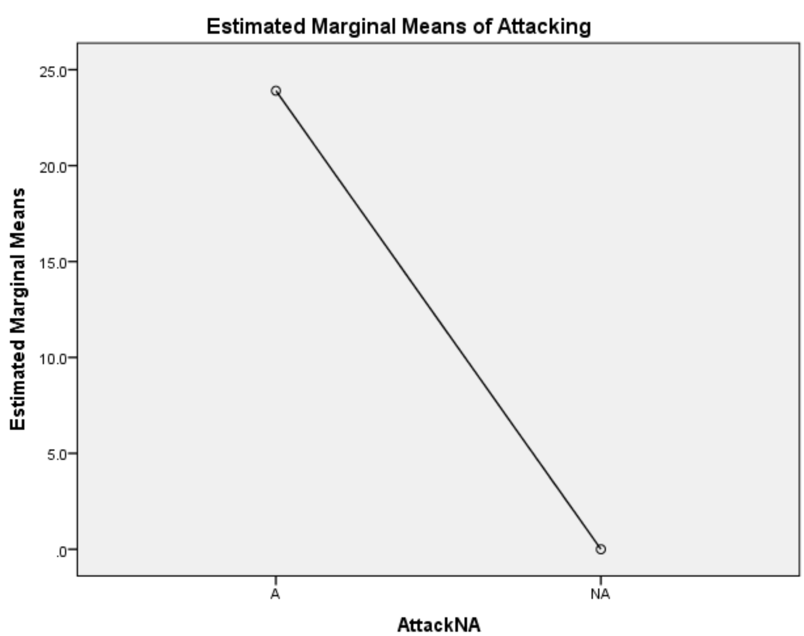

Figure 3: A graph showing the trend of the estimated marginal mean of attacking behaviors in relationship to the presence (A) or absence (NA) of a predator.

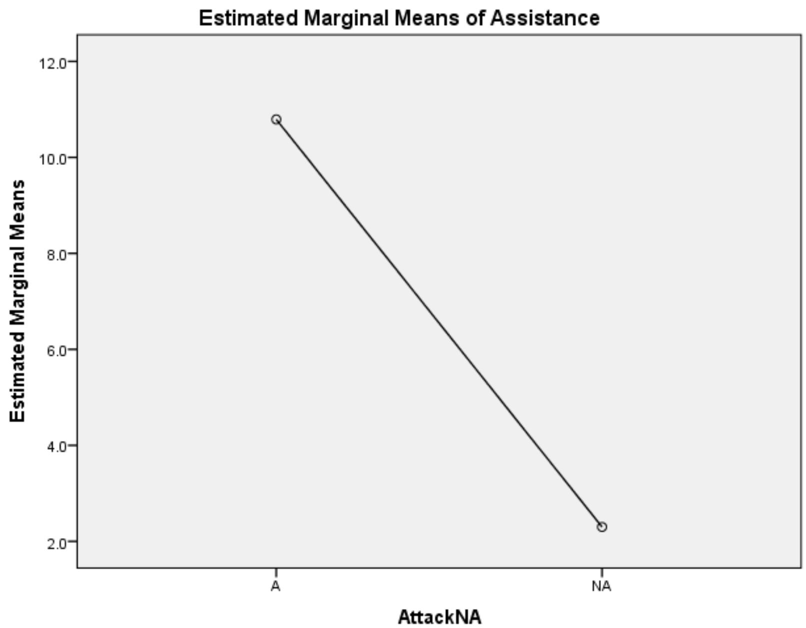

Figure 4: A graph showing the trend of the estimated marginal mean of assistance behaviors in relationship to the presence (A) or absence (NA) of a predator.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
formica2types	Equal variances assumed	.226	.651	-.759	6	.476	-1.7500	2.3049	-7.3899	3.8899
	Equal variances not assumed			-.759	5.863	.477	-1.7500	2.3049	-7.4220	3.9220
lasius	Equal variances assumed	.540	.490	.731	6	.492	1.7500	2.3936	-4.1068	7.6068
	Equal variances not assumed			.731	5.844	.493	1.7500	2.3936	-4.1451	7.6451
camponotus	Equal variances assumed	9.000	.024	1.000	6	.356	.2500	.2500	-.3617	.8617
	Equal variances not assumed			1.000	3.000	.391	.2500	.2500	-.5456	1.0456

Figure 5: An independent samples t-test displaying the distribution of ant species, formica, lasius, and camponotus, in relationship to distance from the road.

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
averagetemp	Equal variances assumed	.000	1.000	-.194	6	.853	-.3750	1.9351	-5.1101	4.3601
	Equal variances not assumed			-.194	5.939	.853	-.3750	1.9351	-5.1220	4.3720

Figure 6: An independent samples t-test analyzing the relationship between temperature and distance from the road.

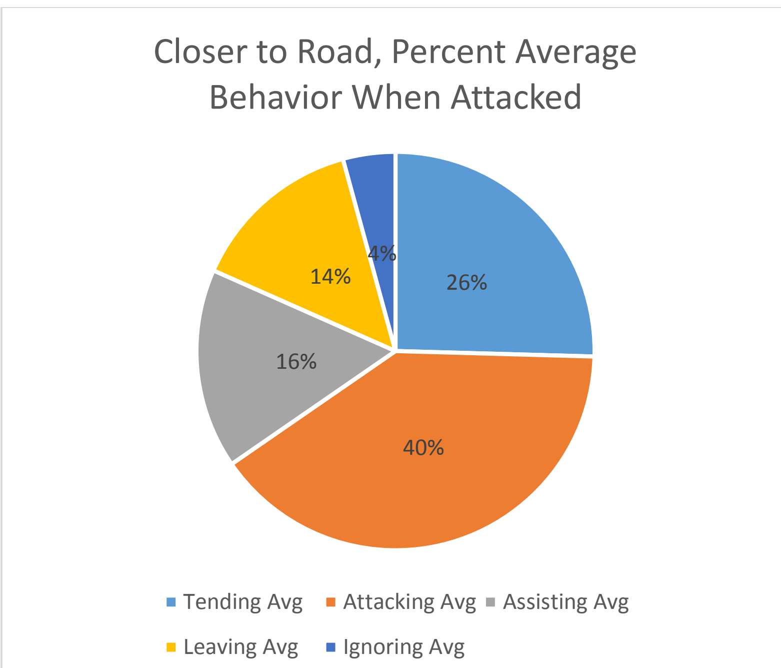

Figure 7: A pie chart showing the proportion of recorded ant behaviors in the presence of a predator on milkweed closer to the road (within 0-10 m).

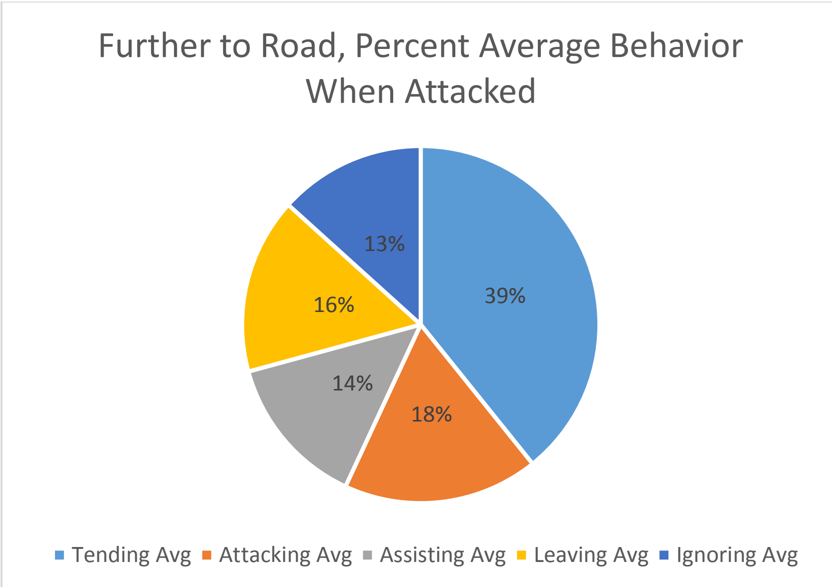

Figure 8: A pie chart showing the proportion of recorded ant behaviors in the presence of a predator on milkweed farther from the road (within 10-20 m).

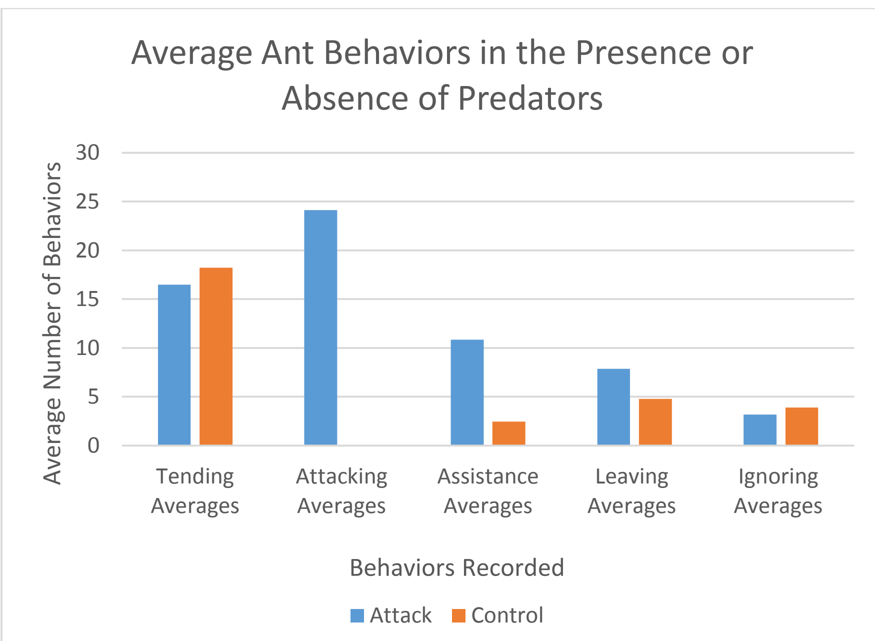

Figure 9: A bar graph displaying the average number of ant behaviors recorded in the presence of a predator (attack) or in the absence of a predator (control).

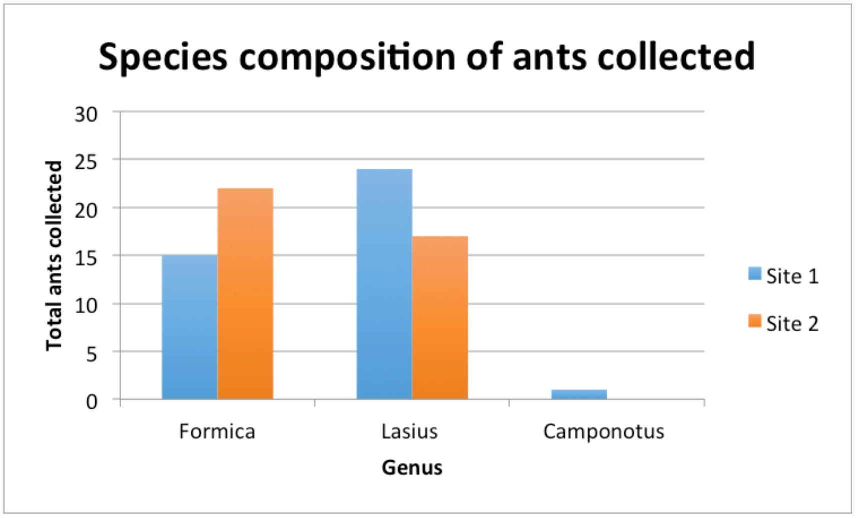

Figure 10: A bar graph displaying the total number of ants collected of each species from the sites closer to the road (site 1) and the sites farther from the road (site 2).

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