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The effect carbohydrate consumption on Argentine ants' nutritional ecology

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The Effect Carbohydrate Consumption on Argentine Ants' Nutritional Ecology

A thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Biology

by

Cheng T Chou

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The Thesis of Cheng T Chou is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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ABSTRACT OF THE THESIS

The Effect Carbohydrate Consumption on Argentine Ants' Nutritional Ecology

by

Cheng T Chou

Master of Science in Biology

University of California, San Diego, 2009

Professor David A. Holway, Chair

As a result of accidental introduction, the invasive Argentine ant, *Linepithema humile*, has successfully invaded many parts of the world including the California coast. Argentine ants are extraordinarily effective in displacing native ants. This study aims to link animal behavior and growth to resource consumption. We examined how different diets affect Argentine ant behavior. We hypothesized that having a diet composed of both carbohydrate and protein may increase colony size and activity level rather than having a diet composed of only nitrogen-based protein. To test this hypothesis, we first conducted a greenhouse experiment in which we used the presence or absence of

honeydew-producing black bean aphids, *Aphis fabae,* to manipulate carbohydrate resources given to the ants. Our results show that carbohydrate-based honeydew gave ant colonies a higher number of surviving workers and a lower trophic level.

We then conducted a lab experiment in which we tested activity and growth potential of Argentine ants from different supercolonies and manipulated their access of carbohydrate. The results showed that having sugar in the diet increased colony activityl, individual level aggression, and likelihood for worker survival, and greater colony growth. These results aid our understanding to the nutritional ecology of Argentine ants, and possibly related species of Hymenoptera.

Chapter 1

Effects on Argentine Ant (*Linepithema humile*) colony size due to mutualistic behavior between aphids and ants

Abstract

Ant-hemipteran mutualism occurs commonly among invasive ants. Argentine ants, *Linepithema humile*, are known to interact readily with many different honeydew-producing hemipteran species in order to gain access to carbohydrate-rich honeydew. To understand the effects of this mutualism between Argentine ants and honeydew-producing hemipterans, black bean aphids *Aphis fabae* were chosen to test the effect of carbohydrate consumption on the Argentine ant's colony size and trophic position, a greenhouse experiment was conducted. After eight weeks, the results show that access to honeydew causes Argentine ant colonies to have a higher worker survival and also a lower nitrogen isotopic level, which is indicative of a lower trophic position. This finding shows that the success of Argentine ant invasions may be influenced by access to carbohydrate resources from ant-hemipteran mutualisms.

Introduction

In the field of nutritional ecology, it is often an intriguing dilemma to find out how dietary macronutrient input affects behavior and in turn population size (Leitzmann 2003, Raubenheimer et al. 2009). What particular macronutrient demand individuals require in order to maximize fitness remains an outstanding question for many organisms. In order to examine linkages between macronutrient demand and population size, a model system would exhibit variation in diet and the factors related to abundance.

Ants are often used as model systems to study such topics (Suarez et al. 2001, Holway et al. 2002). Ants are susceptible to accidental introduction and can become invasive in their introduced range (Suarez et al. 2001). Invasive ants are generalized and opportunistic omnivores; they often consume large amounts of liquid food and readily interact with honeydew-producing Hemiptera (Holway et al. 2002, Lach 2003, Styrsky and Eubanks 2007). The honeydew (a carbohydrate source) produced by Hemiptera could provide an important food source to invasive ants. For example, the yellow crazy ant (*Anoplolepis gracilipes*) disrupts ecosystems on Christmas Island partly due to its interactions with honeydew-producing scale insects (O'Dowd et al. 2003), and the red important fire ant (*Solenopsis invicta*) form widespread mututalistic associations with an invasive mealybug that also provides honeydew (Helms and Vinson 2002).

Research on trophic ecology has been central to the studying of biological invasions (Elton 1958, Diamond and Case 1986, Pimm et al. 1991, Fritts and Rodda 1998, Petren and Case 1998, Holway et al. 2002). Invasive species may compete for dietary resources that result in drop in population size of native species that used to have control over such resources (Elton 1958, Vitousek et al. 1996, Mack et al. 2000).

Understanding dietary resources can provide information on how species introductions influence the structure of trophic structure following invasion. Stable isotope techniques are useful to identify trophic structure (Ehleringer et al. 1986, Gannes et al. 1997, Dawson et al. 2002). An organism's isotopic composition reflects information on what dietary resources were assimilated. One such isotopic level unit that scientists usually measure is nitrogen; especially the relative presence of $^{15}N/^{14}N$. There is another unit that scientists measure: $\delta^{15}N$. Consumers are usually enriched relative to their prey (i.e., predators have a higher $^{15}N/^{14}N$ ratio). Studies on insects generally report a $\delta^{15}N$ enrichment of 2–3‰ per trophic level (Tillberg et. al. 2007). By measuring the ratio of ^{15}N to ^{14}N, and comparing to a worldwide standard (atmospheric N_2), the relative amount of ^{15}N, or $\delta^{15}N$ can be determined, as described below: $\delta^{15}N$ (‰)=($R_{\text{sample}}/R_{\text{standard}}{}^{-1}$)×$10^3$, where *R* is defined as the atomic $^{15}N/^{14}N$ ratio (Costanzo et. al. 2004)..

Argentine ants (*Linepithema humile)* provide an ideal system to test how diets that differ in their macronutrient content (e.g. the presence or absence of honeydew-producing Hemiptera) could affect the colony growth. The Argentine ant has been reported to respond strongly to the presence of honeydew producing insects (Newell and Barber 1913, Way 1963). The Argentine ant originated from Argentina, but due to accidental introduction, is now found in many places in the world today (Holway et. al. 2002). One of such sites of accidental introduction is coastal California, where Argentine ants have replaced the native ants (Holway 1998, Suarez et al. 1998).

Surprisingly, little research has been completed on the relationship between macronutrient availability and abundance or behavior. We have chosen to study the relationship between the availability of honeydew producing Hemiptera and the abundance of invasive ants. We have chosen the Argentine ant and black bean aphids (*Aphis fabae*), an introduced aphid common in California that resides and feeds on black nightshade (*Solanum nigrum*). To test the relationship between different macronutrient availability provided by the presence and or absence of black bean aphids, we conducted a diet-manipulation study. We hypothesize that carbohydrate availability, or aphid presence, will result in a higher abundance (measured in worker's survival) and lower trophic level (measured in lower isotopic level).

Methods

The greenhouse experiment was conduced starting in August to October, 2007 in a greenhouse at the University of California, San Diego Biology Field Station. Argentine ants used in this experiment came from eight different sampling locations in San Diego County, California. A pair of experimental colonies was made from each sample of ants collected. Each experimental colony consists of ten thousand workers, five queens, and roughly equal amounts of brood. Each experimental colony was then randomly assigned to one of two experimental groups: aphids present (n = 8) and aphids absent (n = 8).

In the beginning of the experiment, ants were first introduced into a small plastic container filled with moist, potted soil. The containers were then nested inside a large plastic container (26 X 66 X 40cm). Sixteen potted plants of *S. nigrum* were then randomly assigned to one of these large plastic containers. The sixteen potted plants were then randomly assigned into either of the two experimental groups, but in general, similar sized potted plants were assigned to each pair of experimental colonies. For the eight potted plants that were assigned to aphids present experiment, *A.f. solanella* was introduced immediately. Ants eventually moved from the small container into the potted *S. nigrum*. To prevent ants from escaping, the large containers were lined with Fluon and balanced on pedestals in bowls of water. Due to the overpopulation of aphids and the presence of spider mite *Tetranychus urticae*, the potted *S. nigrum* died in every experimental colony. Whenever this happened, the dead *S. nigrum* was then replaced with a new one. Each potted plant was watered every 2 to 3 days, given 1 frozen cricket for 3 or 4 days, for aphids present experimental groups, the aphids were replenished once a week, but any aphids colonizing the aphid absent plants were removed, and *T. urticae* also removed whenever they were found. Other than the crickets *Acheta domestica* and the aphids, no additional food was provided.

After 8 weeks, ant colonies were sacrificed to determine the number of workers present (colony size).. Paired t-tests were used to compare mean difference in size of worker ants between aphid present experimental groups and aphid absent experimental groups.

To calculate $\delta^{15}N$, we used methods similar to those described in Tillberg et al. (2006, 2007). The samples from the same experimental colonies were weighed to approximately 1500 μg. Only head, thoraces, and legs were used in sampling. We froze the ants, collected them into vials, and then dried the samples to around 50 degrees Celsius. Until the samples got processed, they were held in a sealed container with desiccant. We weighed all samples into tin capsules and used a Mettler & Toledo microbalance to estimate mass. Analysis of nitrogen isotopic ratios was performed at the University of California, Davis, Stable Isotope Facility using a Europa Hydra 20/20 continuous-flow IRMS.

For each of the eight pairs of experimental colonies and for each time period for which we sampled ants, we subtracted the $\delta^{15}N$ value of workers without aphids from that of workers with aphids. We determined trophic fractionation of ^{15}N in *L. humile* by comparing the $\delta^{15}N$ of the colonies to the $\delta^{15}N$ of the diet for colonies reared on each treatment.

Results

After eight weeks workers from colonies with access to honeydew-producing aphids had lower $\delta^{15}N$ values compared to workers from colonies that did not have access to aphids (Fig. 1.1.A; paired t-test: t7 = 2.60, p = 0.035). We subtracted the $\delta^{15}N$ value of workers without aphids from that of workers with aphids

Also, compared to colonies with no access to honeydew-producing aphids, colonies with aphids had higher numbers of surviving workers at the end of the experiment (Fig. 1.1.B; paired t-test: t7 = 4.27, p = 0.0037). We took the difference between aphid absent colonies and aphid present colonies. A negative number meant the aphid present colonies had more surviving workers.

Discussion

From the result, we have demonstrated that the presence of honeydew-producing aphids lowers the trophic level of Argentine ants along with increased abundance through increased worker survival. Our study provides experimental support for the hypothesis that carbohydrates from honeydew producing Hemipterans could result in increased ant abundance in nature. The nitrogen isotopic data further provide support by showing that ants with aphids do have a lower trophic level compare to the ones with only insect prey for food source.

Previous studies have shown that having access to carbohydrates (as compared to having primarily protein) would contribute to the ability of Argentine ants to become behaviorally dominant (Grover et al. 2008); our study provides further support that having the carbohydrate food source from presence of honeydew-producing aphids would result in Argentine ants' greater abundance in the introduced range.

Relatively lower trophic level often equates to higher population size. From the experiment, we find that there are more surviving workers from the colonies that also have lower trophic level. It is intriguing to see how one factor is related to the other. Normally one would assume that individuals with higher trophic level should be more likely survive given that they are further up on the food chain; yet, that is not what we observe.

From the results of this experiment, we could possibly learn how to deter Argentine ants. While we have not been able to find a natural enemy that could compete successfully with Argentine ants (Tsutsui et. al. 2001, Wild 2004), we could possibly test how increasing honeydew-producing would Hemiptera might affect the Argentine ant colony growth. Increased a predators of Hemipter would ultimately drive down the number of Hemiptera, and in turn, drive down the number of Argentine ants. Increased Hemiptera predators would also make the Argentine ant increase their predation on these insects, which is a higher nitrogen-based diet compare to the honeydew, so that would increase the trophic level of the Argentine ants.

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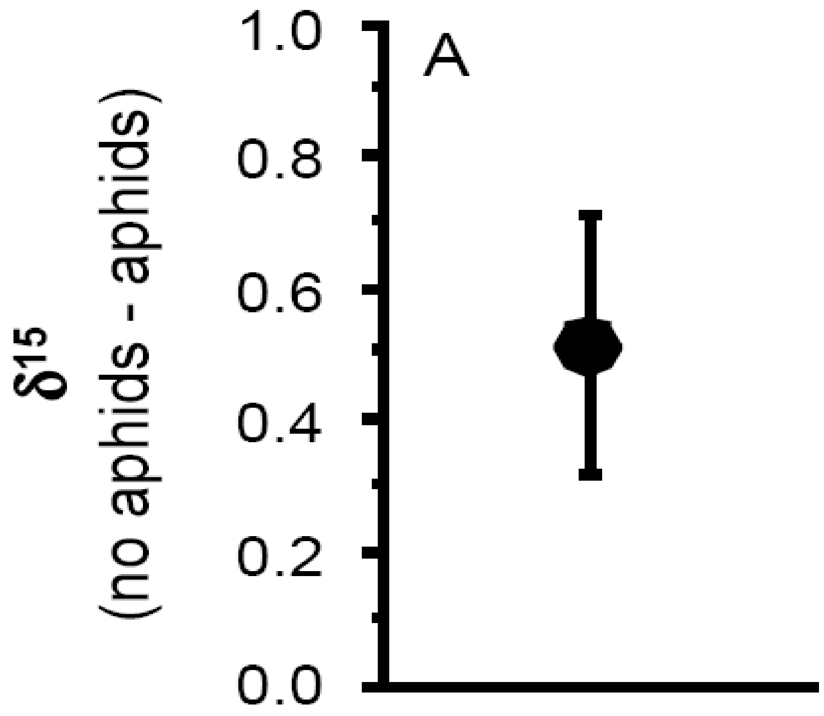

Figure 1.1.A. *Different in nitrogen isotopic level***.** Colonies with access to honeydew-producing aphids had lower δ15N values compared to workers from colonies that did not have access to aphids.

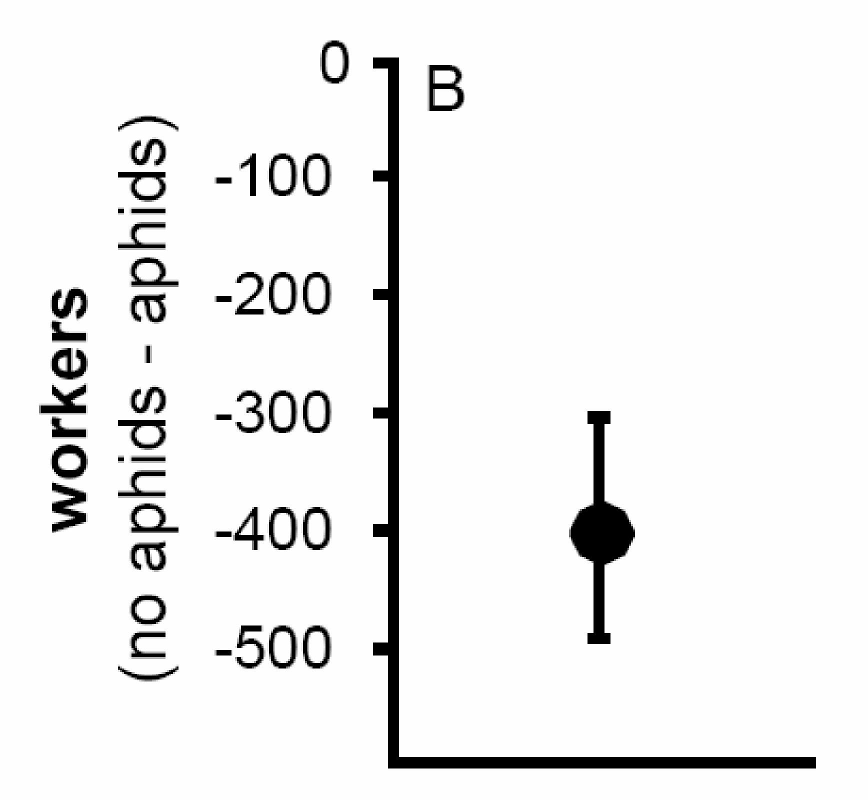

Figure 1.1.B. *Different in workers survival***.** Colonies with aphids had higher numbers of workers at the end of the experiment

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Chapter 2

Effect of carbohydrate availability on Argentine Ant (*Linepithema humile*) activity level and colony size

Abstract

Ant invasions are detrimental phenomena now found in many parts of the world. Here in California, Argentine ants (*Linepithema humile*) displaced many native ants. Following the discipline of nutritional ecology, the successful invasion of Argentine ants may be linked to the diet they consume. To study how different diet could affect the Argentine ant's activity, aggression, and colony growth (e.g., brood production and worker survivorship), a diet manipulation experiment was conducted. Here, we have found that having access to carbohydrates increases the colony activity level and the aggression level of individual ant. However, the effect of exploration ability and battle surviving ability is not significantly different from the effects of diet. Also, it was found that having access to sugar increases worker survival and also colony growth. These data show that having access to carbohydrate resources may increase Argentine ant fitness, and could caused their success in invasion.

Introduction

In the field of nutritional ecology, scientists assume that population size and behavior are both related to dietary input (Leitzmann 2003, Raubenheimer et al. 2009). Different macronutrient intake could affect behavioral ecology and fitness. In this study we asked why the absence or presence of a particular macronutrient could result in difference in behavior and population size. To study this ecological phenomenon, we carried out a diet manipulation study using the Argentine ant as our model system.

Invasive ants provide evidence of how dietary macronutrient intake contributes to differential behavior (Holway et al. 2002). Honeydew producing Hemiptera provide invasive ants with plant-based carbohydrate rich food source, and the invasive ants' high activity level and aggressive behaviors seem directly linked to the consumption of such liquid food (Newell and Barber 1913, Way 1963, Helms and Vinson 2002, O'Dowd 2003). The Argentine ant is an introduced species found in coastal California. This species has shown a broad trophic position. They consume carbohydrates wherever they are found (Tsutsui et al. 2001, Holway et al. 2002, Menke and Holway 2006).

Argentine ants in California have reduced genetic variation and have formed a giant "supercolony" of closely related ants (Tsutsui et al. 2000). Part of the reason that Argentine ants are successful in California may be due to reduced intra-specific competition among the Argentine ant colonies. Argentine ants do fight different super colonies. The Large Supercolony currently takes control of the vast area along the coast line that stretches from San Diego all the way to San Francisco, and there are several small pockets of Argentine ants of different supercolonies on the inland part of San Diego County (Holway and Case 2001).

To study how nutrition would affect the behavior and population size of the Large Supercolony, we set up lab experiment to study the effect of diet. We manipulated diet with equal amount of caloric value but with presence or absence of carbohydrate in order to see if carbohydrate availability has any effect on individuals and colony level. We hypothesize that having access to carbohydrates will increase Argentine ant's activity level and colony size.

Methods

Starting in May 2008 we collected material of experimental colonies for Argentine ants from various locations in San Diego County, CA. Ants from two different supercolonies were collected: one from the "Large Supercolony," and the other from the "Lake Hodges Supercolony." For the Large Supercolony, ten sites were collected, each one being at least two hundred meters away from the others. Ant colonies from each site were used to produce two experimental colonies. For the Lake Hodges Supercolony, total of twenty experimental colonies were collected from that one single site. The experimental colonies were set up in a similar method as in Grover et. al. (2007). Each experimental colony consisted of one thousand workers, five queens, and an approximately similar amount of brood. The colonies were reared in circular plastic containers (28 diameter x 10 cm height) lined with Fluon to prevent ants from escaping. All of the containers had a hole drilled on the side. Each container had four nest chambers made up of glass test tubes (16 x 150 mm) filled half full of water and stopped with cotton. The glass tubes were covered with aluminum foil to maintain the dark environment preferred by the Argentine ants. The colonies were reared in our laboratory with 12:12 hour light/dark cycle and approximately constant temperature at 20 degrees Celsius. Before the start of the experiment, the ant colonies were fed 10% sucrose solution and domestic crickets (*Acheta domestica*) *ad libitum*.

The set up of the experiment was to pair up two experimental colonies from the Lake Hodges Supercolony with two experimental colonies from the same Large Supercolony collection site. We call one of these 4 experimental colonies match-up a "group." There are ten groups total. For each group, one Large Supercolony colony fed with both sugar and protein (coded as AL+) will be linked to one Lake Hodges Supercolony fed with only protein (AH-), and one Large Supercolony fed with only protein (BL-) will be linked to one Lake Hodges Supercolony fed with both sugar and protein (BH+). Each experimental colony had a 30cm long plastic tubing inserted in the hole mentioned earlier on the container at the start of the experiment; the tubing was connected with two layers of fine mesh in the middle. The purpose was to allow ants from the different supercolonies to be able to detect each others' presence, yet prevent them cross over and interact. The mesh were observed on a daily basis, if any small holes were made, the mesh were changed right away.

As mentioned, two different diets were given: the sugar and protein diet versus the protein only diet. The protocol for making the two diets is the same, the difference is the amount of ingredients used. To make the food, we poured liquid nitrogen over frozen crickets, and then grinded them using mortar and pestle until the crickets turn into a relatively homogenous powder. One hundred-fifty milliliter of water and 1.5 grams of powderized agar were used in every batch of food. The crickets are roughly equivalent to 24% protein with 60% protein wet mass and 40% dry mass; thus, for the protein only diet, we added 24 grams of crickets to the water and agar, and for the sugar and protein diets, we added 2.9 grams of sugar and 12.1 grams of crickets. We followed this recipe to ensure that we maintain an equal caloric content in both diets. About 1 gram of agar gel was fed to each colony daily, and the ants were fed 5 or 6 times a week.

On a daily basis, we counted the number of ants located on or around the mesh to measure activity level of the ants. We continued doing so until the end of the experiment. This behavioral assay was done by taking apart the connective tubing and count carefully the numbers of ants on both side of the meshes. Also, on a bi-weekly basis, we have conducted a one-on-one battle to measure the following: exploration ability, aggression behavior, battle survival. Two kinds of match-ups were deployed: match-ups between the AL+ versus AH- and match-ups between AH+ versus AL-.

For this behavior assay, two fluoned Petri dishes, each containing one ant from the respective treatment, were linked together by a small bridge. We first recorded the time when one ant first crosses over the bridge, then we removed the bridge, and recorded the time again when one of the ant showed aggressive behavior to the other. Lastly, we recorded the survivor of the battle. This test was done blind, meaning the person who was recording and judging how the ants behave would not know which treatment the specific ant came from..

The experiment ran for fourteen weeks. By the third to last week of the experiment, we tested how diet affected the colonies ability to fight as a whole. We lifted the mesh and allowed the connected colonies to contact with each other. From previous studies and the one-on-one battles behavioral assays, we were sure that war would break out between the two supercolonies. The battles were all ended after one week of exposure.

Colonies fed sugar and protein were able to expand into their enemies' container. We carefully removed those ants back in their original container, and then froze all colonies. We then counted the number of workers, queens, eggs, and larvae under microscope to see how as a whole the diet had influenced the colony size.

Results

On the colony level, activity was assessed through mesh count activity. We took the difference between the average number of ants on the mesh from all 10 of groups of supercolonies and diet to the average number of ants from their 10 match-ups (Figure 2.1.A). From paired t-tests it was shown that the difference between sugar treatments and no-sugar treatments were significant: For the AL difference, which has the large supercolony with sugar treatment minus the Lake Hodges supercolony with no sugar treatment (T value = 5.232 with P-value <0.0001) and for the BL difference, which has large supercolony with no sugar treatment minus the Lake Hodges supercolony with sugar treatment (T-value = -5.048 with P-value <0.0001). Colonies with sugar treatments are much more active.

On an individual level, we found no significant difference in exploration ability between the two different diets (Figures 2.2.A, T-value 0.913, P value= 0.3964), Large Supercolony ants fed with sugar had ants that were more aggressive compared to the Large Supercolony ants fed no sugar (Figure 2.2.B, T-value = -6.942, P value = 0.0004), and there was no significant difference in survival ability between the two treatments (Figure 2.2.C, T-value = -.4272, P-value = 0.6841).

As for abundance, colonies with sugar in their diet had higher numbers of works at the end of the experiment (Figure 2.3.A, Pair A, mean difference = 277, t9 = 4.96, p-value = 0.0008; pair B, mean difference = 401, t9 = 9.95, p-value = <0.0001). Also, Colonies with sugar in their diet had higher numbers of reproductive possibility, calculated by adding up the combined number of eggs and larvae in the colony, at the end of the experiment (Graph 2.3.B). (Pair A, mean difference = 156, t9 = 5.51, p-value = 0.0004; pair B, mean difference = 102, t9 = 6.91, p-value = <0.0001).

Discussion

From Figure 2.1.A, the effect of carbohydrates on Argentine ant colony activity level was obvious. The difference in mesh count represents different activity levels of the colonies as a whole. From the figure, it is apparent that the presence/absence of carbohydrate hugely affects difference in activity.

Figure 2.2 show the difference in behavior caused by diet manipulation on individual level instead of on a colony level. Figure 2.2 is only focusing on Large Supercolony. Figure 2.2.A shows that there is no difference between the ability of exploration between ants of the different macronutrient uptake. Both sugar and protein treated ants show a similar probability of being the one to first cross the bridge and explore unknown territory. Figure 2.2.B shows that there is a significant difference in behavioral dominance due to having difference macronutrient uptake. The sugar treated ants are always more likely to be the ones to first show aggressive behavior when the two ants from different super colony meet up in the Petri dishes. This result further supports the same conclusion from Grover et. al. (2007). Lastly, Figure 2.2.C shows a rather intriguing case. Base solely on the graph, it shows that during the first half of the experiment, the sugar treated ants were actually less likely to survive the battle; but during the second half of the experiment, the sugar treated ants were more likely to survive the battles. Our only assumption was that there's a possibility of the protein treated ants, initially lacking of carbohydrates, increased their activity in hope of finding carbohydrates source.

Figure 2.3 shows the survival worker count and reproductive possibilities of the colonies at the end of the experiment. Figure 2.3.A shows the average number of worker counts. Both pair A and pair B have a positive average number, which tells us that the colonies fed with sugar were always more likely to survive the final colony-to-colony battle that took place on the final stage of the experiment. Graph 2.3.B shows the average number of reproductive possibilities that is calculated by adding the combined number of eggs and larvae in the colony. Both pair A and pair B have a positive number again. Colonies fed sugar are always more likely to have a higher reproductive possibility. The second result seems surprising because most of the workers in the protein only colonies couldn't even the final battle.

One complication the experiment is the effect of the diet itself on ant survival. Throughout the experiment, it was noticed that protein treated colonies always had higher work mortality compare to the sugar treated ones. The protein diet on some level is related to the Atkins diet. Atkins diet is comprised of high protein and low carbohydrate meals (Foster et. al. 2003). People who consume Atkins diet were likely to develop ketonacidosis, a problem in which the body cumulates excess ketones, which acidifies the body and create health issues. Furthermore, by-products of ketones will cumulate in kidneys, creating kidney-problems, and causing detrimental damage to the body (Foster et. al. 2003). There are currently no biochemical studies on over consumption of protein diet in ants, but this is one estimated result that could have taken place. Nevertheless, whether the differential worker counts is caused by the diet itself, or the behavior that resulted from the diet, the manipulated diet is still the reason for the difference in surviving workers.

From this study, we have found that having carbohydrate for food will increase activity level on both individual level and colony level in Argentine ants. Also, after long term of diet manipulation, the presence of carbohydrates in diet will certainly increase the survivorship probability and reproductive possibility for Argentine ants. Similar result is also likely to be found in other species of ants, or perhaps other species of Hymenoptera. One of the other source of macronutrient, lipid, wasn't being taken account in this study, perhaps future studies could utilize lipid into account in another diet manipulation study to see how would the presence/absence of lipid could affect ants activity level and survivorship.

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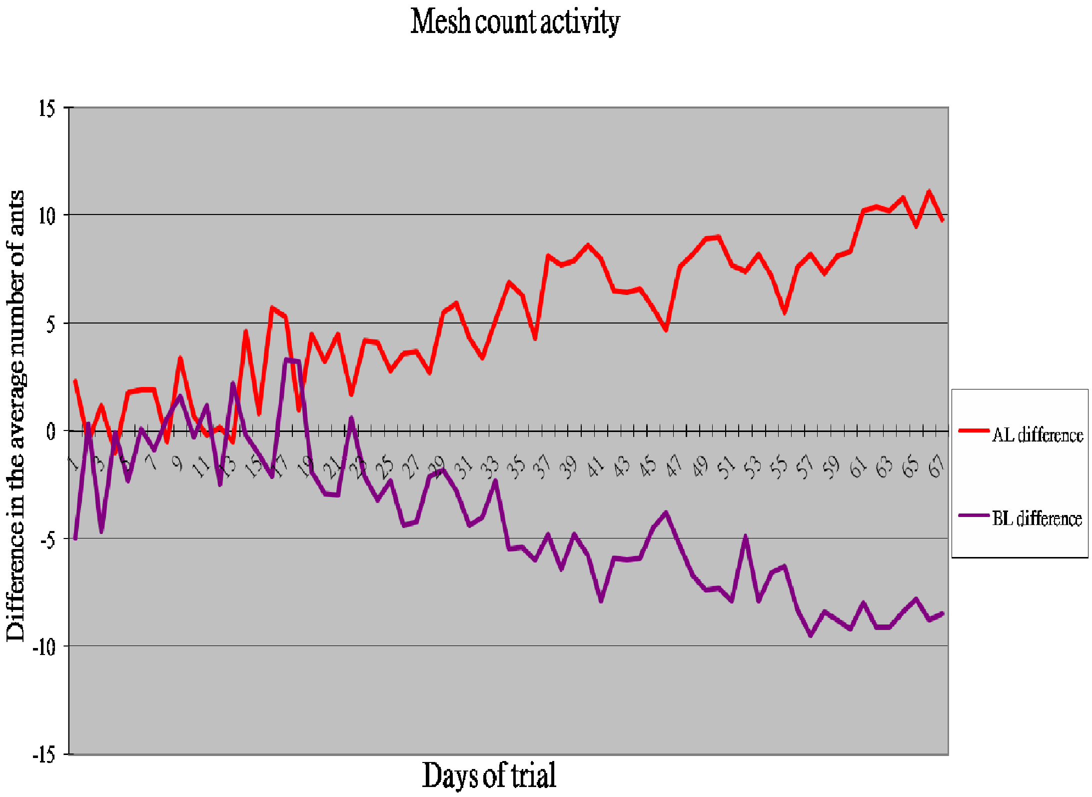

Figure 2.1.A. *Difference in mesh counts.* The difference between sugar treatments and no-sugar treatments were significant with colonies having access to sugar being more active.

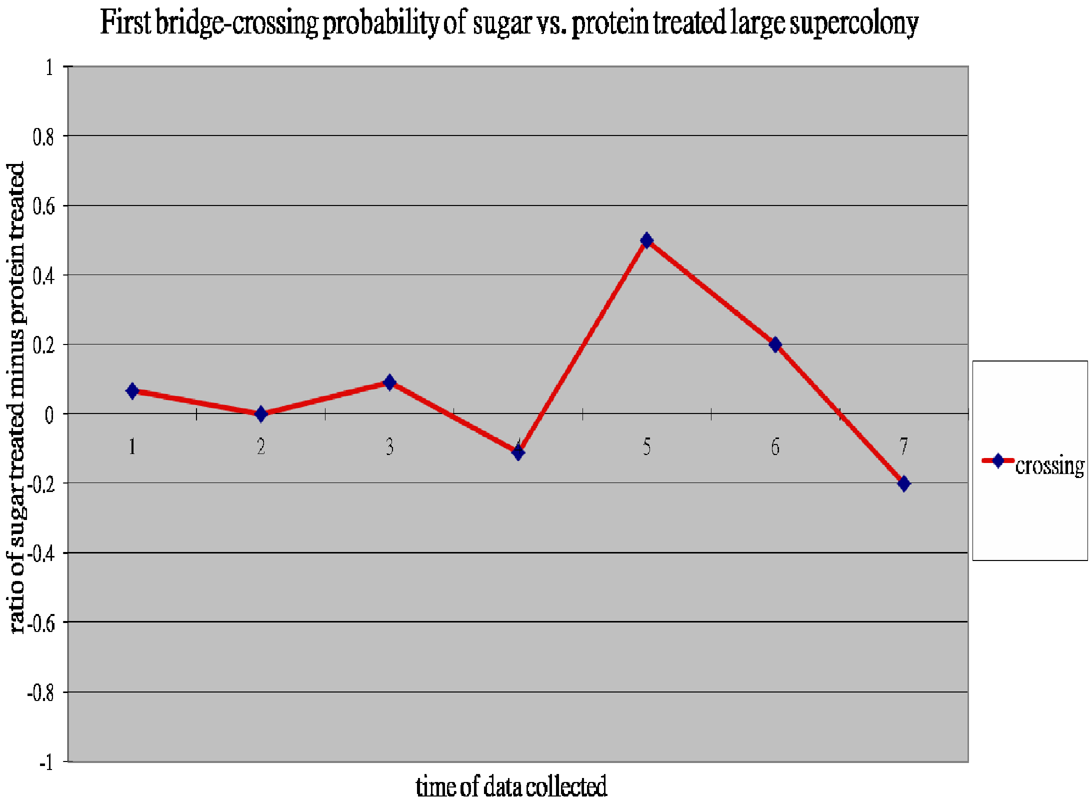

Figure 2.2.A. *Difference in exploration ability.* There was no significant difference in exploration ability among the two different diets (T-value 0.913, P value= 0.3964)

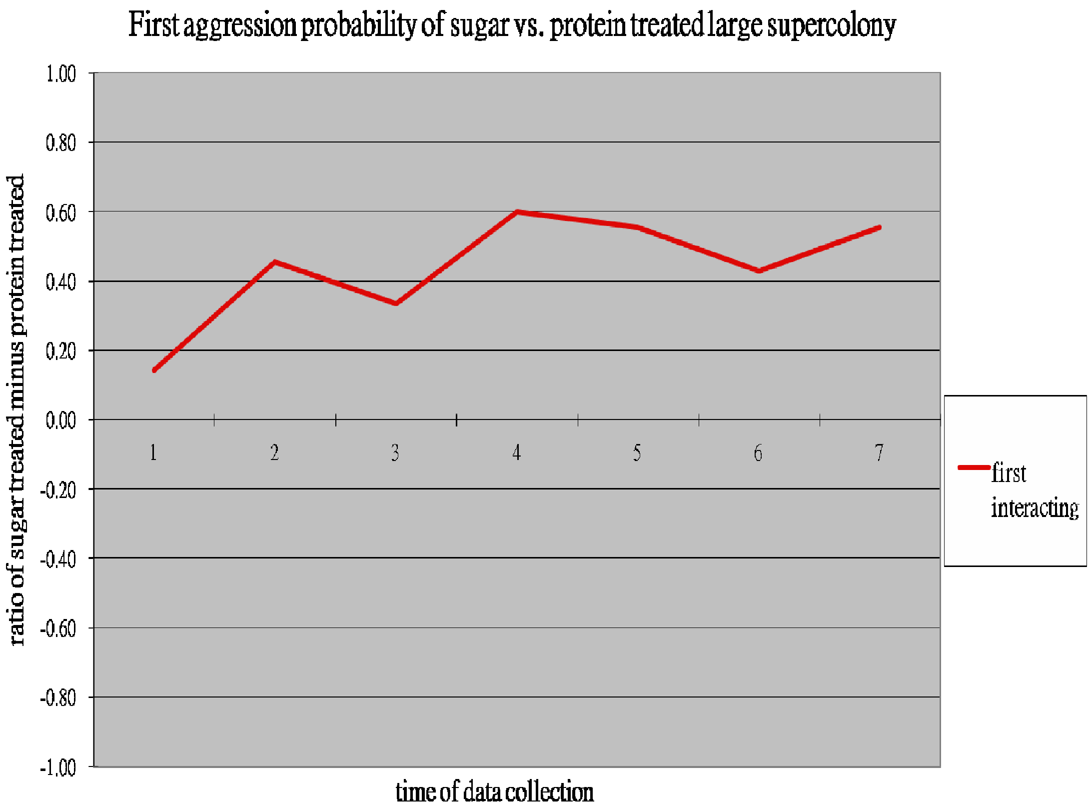

Figure 2.2.B. *Difference in aggression level.* Large Supercolony ants fed with sugars had ants that are more likely to initiate aggression compared to the Large Supercolony ants fed with no sugar (T-value = -6.942, P value = 0.0004)

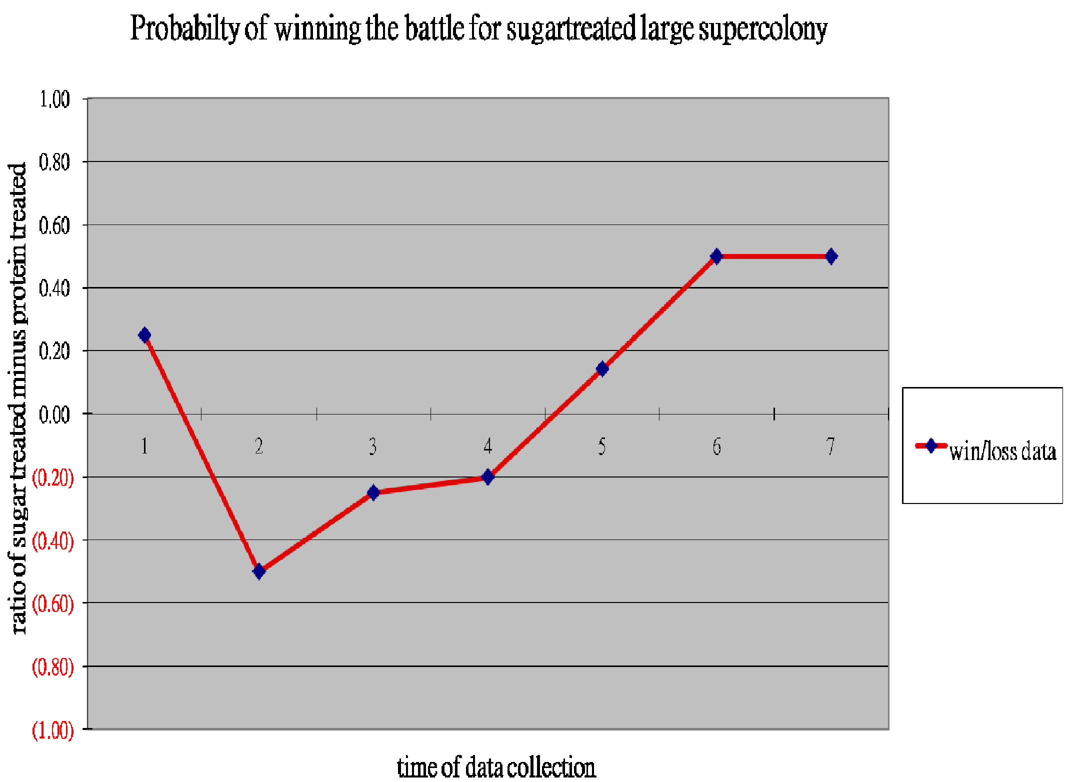

Figure 2.2.C. *Difference in survival.* There is no significant difference in survival ability among the two treatments (T-value = -.4272, P-value = 0.6841).

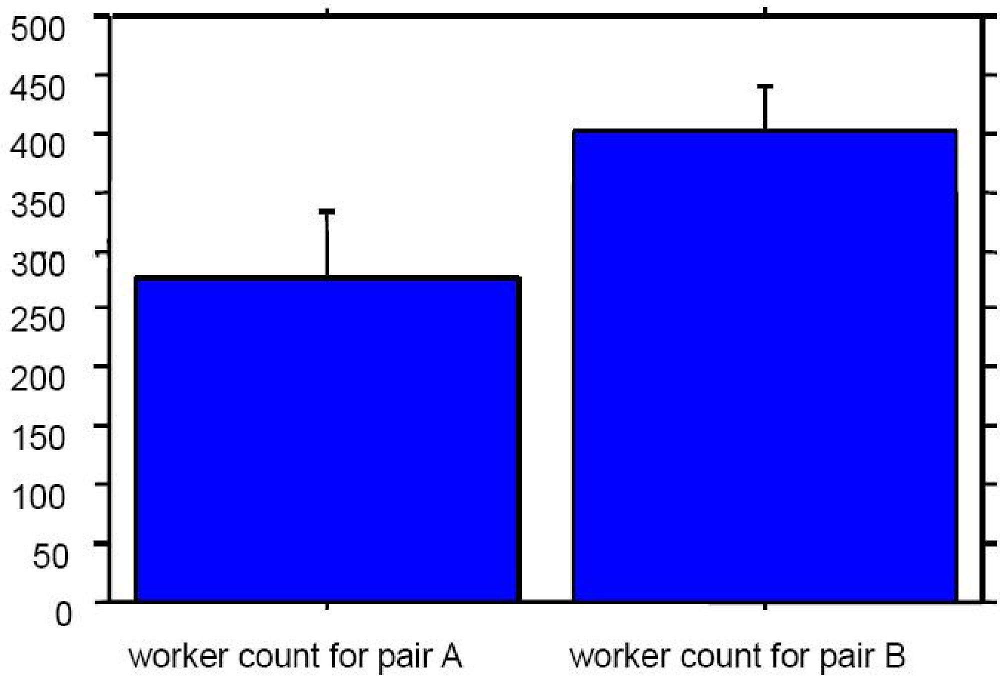

Figure 2.3.A. *Difference in worker survival.* Colonies with sugar in their diet had higher numbers of workers at the end of the experiment. (Pair A, mean difference = 277, t9 = 4.96, p-value = 0.0008; pair B, mean difference = 401, t9 = 9.95, p-value = <0.0001)

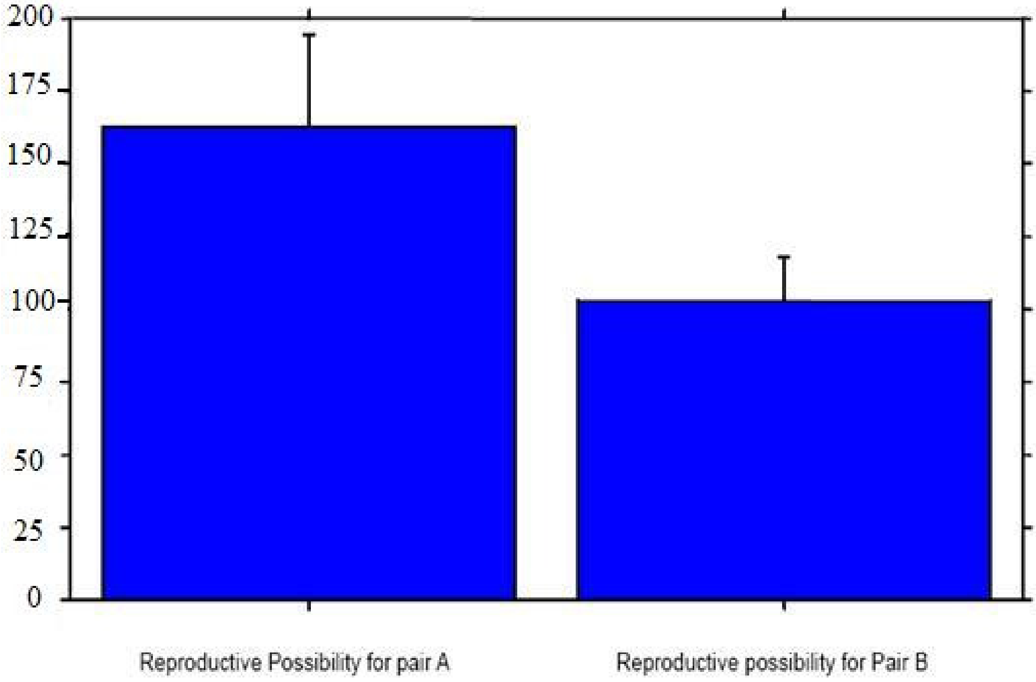

Figure 2.3.B. *Difference in reproductive possibilities.* Colonies with sugar in their diet had higher numbers of reproductive possibility at the end of the experiment. (Pair A, mean difference = 156, t9 = 5.51, p-value = 0.0004; pair B, mean difference = 102, t9 = 6.91, p-value = <0.0001).

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