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Crystal Ramirez
Department of Environmental Studies, University of California, Santa Barbara
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ABSTRACT

Leafcutter ants *Atta cephalotes* (Hymenoptera: Formicidae), common to Central and South America, have a profound caste system to ensure the cultivation of a special fungus, *Leucocoprinus gongylophorus*, which is their main food source. Within the caste system, the workers job is to forage and carry back pieces of leaves that are necessary for fungus farming. The minima have long been thought to be protecting the worker ants from parasitic phorid flies (Diptera: Phoridae), who have been studied to await near the entrance of the nest to land on a leaf and lay eggs on the head of the worker ant. But, there have been various studies that have suggested that hitchhikers have other functions. The other two hypotheses include cleaning the leaf and feeding from the sap. Using four methods and previous student research, I tested and analyzed the most probable function of the minima. On average, there were more hitchhikers present near the entrance of the nest than there were 6 meters away. The hitchhikers also showed to perform multiple behaviors along the trail and are most likely to react protective due to a disturbance on the leaf. Lastly, the hitchhikers ride the leaf for a long distance until the entrance of the nest, or for a short distance and get off the leaf the leaf before getting to the entrance. My study concludes that hitchhikers perform multiple functions depending on various factors, such as the time of day and the location of the trail.

Estudio detallado de la función de las mínimas en hormigas cortadoras de hojas (*Atta cephalotes*)

RESUMEN

Las hormigas cortadoras de hojas *Atta cephalotes* (Hymenoptera: Formicidae), comunes en América Central y del Sur, tienen un complejo sistema de castas para asegurar el cultivo de un hongo especial (*Leucocoprinus gongylophora)*, que es su principal fuente de alimento. Dentro del sistema de castas, el trabajo de las obreras es forrajear y transportar segmentos de hojas que son necesarios para cultivar el hongo. Durante mucho tiempo se pensó que las hormigas mínima protegían a las hormigas obreras de las moscas parásitas (Diptera: Phoridae), que se conoce que esperan cerca de la entrada de la colonia para aterrizar sobre una hoja y poner huevos en la cabeza de la hormiga obrera. Sin embargo, varios estudios han sugerido que las mínimas tienen otras funciones. Las otras dos hipótesis incluyen: limpiar las hojas y comer savia. Usando cuatro métodos propios e investigaciones previas de estudiantes, probé y analicé la función más probable de las mínimas. En mi estudio, hubo en promedio más mínimas

presentes cerca de la entrada del nido que a 6 m de distancia. Las mínimas también mostraron comportamientos múltiples a lo largo del camino y son más propensos a reaccionar como protectoras cuando hay una perturbación en la hoja. Por último, las mínimas pueden montar la hoja en una larga distancia hasta en el nido, o montarla por una corta distancia y bajarse de la hoja antes de llegar al nido. Con este estudio concluyo que las mínimas cumplen diferentes funciones dependiendo de varios factores como la hora del día y la ubicación en el sendero.

The term eusocial is used to describe animals that show complex behaviors, which is the highest level of organization of sociality. Usually, eusocial animals include one reproductive female and several unbreeding individuals that perform specific jobs. There are few animal groups that are eusocial, one being the leafcutter ants. Leafcutter ants of the genus *Atta cephalotes* are abundant in tropical rainforests and tropical dry forests in elevations below 2,000 meters. A leafcutter colony is one of the most interesting ants because their caste system is well organized for the cultivation of fungi, which is their main food source. The production of the fungus requires the colony to have an advanced eusocial structure that entails every caste level to carry an important role to ensure that the colony is fed.

One leaf cutter ant colony consists of a queen ant with the sole purpose of laying eggs. The soldiers are the larger ants that are usually seen in the entrance of the nest to protect the colony from any predators. The foragers (or carriers) who are seen in the trails carry leaves back to the nest. There are also different caste levels for the nursers of leaves and pupae, the constructors of chambers and the handlers of trash. The caste system also contains minimas, most commonly known as hitchhikers for riding the leaves that the foragers are carrying.

The hitchhiker's function is not completely known, but the most well-known hypothesis is the protection of the carrier ants from parasitic phorid flies. These parasitic phorid flies prefer to lay their eggs on the heads of carrier ants, which eventually kills the carrier ant and becomes a threat to the whole colony (Holbrook et al., 2014).

There are also other studies that suggest that the hitchhikers have other functions. The Linksvayer (2002) study concluded that there are multiple possible hypotheses for the hitchhiker's function. For the purpose of my research I will be further examining three of the hypotheses that make the most sense. (1) The hitchhikers are protecting the carrier ant from parasitic phorid flies, (2) the hitchhikers are cleaning the leaf by removing microbial contaminants, and (3) the hitchhikers are feeding on the leaf sap.

The purpose of my study is to research which of the hypotheses is most supported depending on the behavior of the hitchhikers. I tested the hypothesis that hitchhikers are more active near the entrance of the nest with their heads up ready to attack flies and less active farther away from the entrance of the nest with their head down where phorid flies are not as active, which supports the protecting the carrier ant hypothesis (Elizalde and

Folgarait 2012). I also hypothesize that if more hitchhikers are observed with their heads up or moving on the leaf then it supports the protecting the carrier ant hypothesis. And if more hitchhikers are seen with their head down then it supports the cleaning the leaf and eating sap off the leaf hypothesis. I also tested the hypothesis that more phorid flies will go towards a disturbance on the leaf. And, lastly, I hypothesized that hitchhikers will ride the leaf for short distances which will support the eating the sap hypothesis.

MATERIALS AND METHODS

I studied five *A. cephalotes* colonies from May 10 through May 18 of 2019. Four of the colonies observed were located in La Calandria and the last colony was observed in Finca Paraíso in Los Tornos. I counted the total number of carrier ants and the number of hitchhikers along the trail to test if hitchhikers are more protective near the entrance of the nest. To study the hitchhikers position on the cut leaves assuming a defensive or not defensive position, I observed five different leafcutter colonies and collected data on the head position of the hitchhikers: facing upwards towards the sky, facing downwards towards the cut leaf or moving around the leaf. I collected data on the reaction of the hitchhikers after a leaf disturbance to test whether the hitchhiker would react protective. To analyze the third hypothesis, the ingestion of sap, I recorded the travel distance of hitchhikers for various colonies in La Calandria.

Hitchhiker Rate

For each of the five colonies observed, I took data on the distribution of hitchhikers along the trails. For 2 minutes, I counted the number of leafcutter ants and the number of hitchhikers present for the entrance of the nest and 6 meters away from the nest. This data will help interpret the frequency of the hitchhikers.

Head Position

The data I collected for the head position of the hitchhikers was taken near the entrance of the nest and also 6 meters away from the entrance of the nest for the five colonies. The amount of data I collected was determined on how active the colony was. If the colony was active I was able to collect 63 data points, but if the colony was not as active I collected data for an hour. The hitchhikers observed were either still with their head facing the leaf, still with their head facing up away from the leaf or moving around the leaf with their head down facing the leaf. The head position of the hitchhiker will help determine which hitchhiker function hypotheses is most supported. Hitchhikers with their head up will support the hypothesis in favor of protection of the carrier ant from phorid flies. The head down position and the moving around the leaf with head down will support the eating sap and cleaning the leaf hypotheses.

Leaf Disturbance

I tested whether the hitchhikers are protective by disturbing the leaf with a pencil. At random, I chose a leaf that had a hitchhiker and placed my pencil on top of the leaf to try to stimulate a phorid fly perching on the leaf before attacking the carrier ant. Data was categorized into three sections that the hitchhikers responded as: the hitchhiker would either go towards the object, off the leaf, or it would not react to the object on the leaf.

The results will help determine whether the hitchhikers are protecting the carrier ants from parasitic phorid flies.

Travel Distance

Ten random leafs with a hitchhiker were followed to observe the distance that hitchhikers ride the leaf. Starting from 3 meters away from the nest I chose a leaf to follow and recorded the distance from the 3 meter mark that the hitchhiker got off the leaf. This data will help determine whether the hitchhikers are eating the sap from the leaf. If the hitchhiker is on the leaf for a long distance it will not support the hypothesis because the hitchhiker would most likely not continue feeding for a long period of time, especially since their weight can make it harder for the carrier ant to carry the leaf back to the colony.

RESULTS

I found that there were 26% more hitchhikers observed in the entrance of the nest than 6 meters away from the entrance (Figure 1). The hitchhiker behavior and the location of the trail did not have a significant difference as the percentages are all close in range (Figure 2). I also found that more hitchhikers respond ready to fight with a leaf disturbance by going towards the object on the leaf (Figure 3). There is a statistical significant difference between hitchhikers going towards the object than hitchhikers getting off the leaf or not reacting (x^2=38.57, $p<0.05$). Table 1 also shows that not all hitchhikers ride the leaf into the nest and can get off of the leaf at any distance on the trail. With the data I collected, the hitchhikers either got off the leaf close to 3 meters away from the nest or rode the leaf into the nest. Few hitchhikers got off the leaf midway through the trail (Table 1).

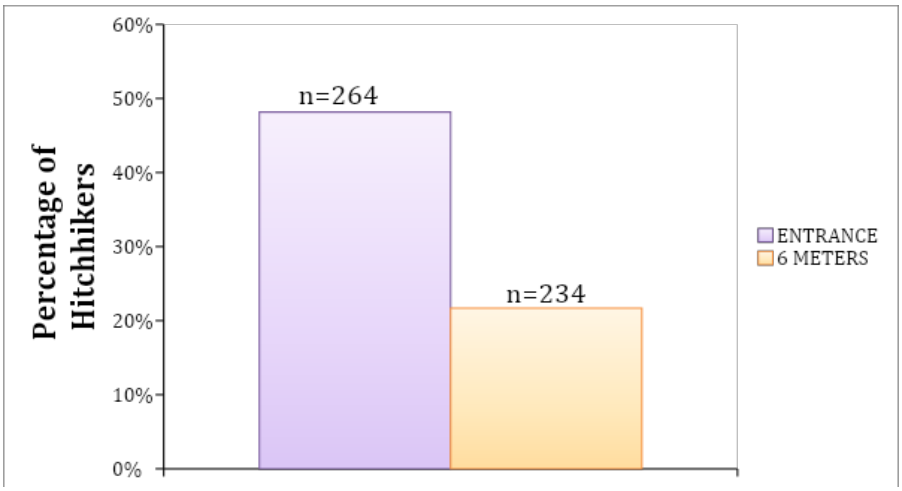

Figure 1. Percentage of hitchhikers observed on the leaves carried by the carriers near the entrance of the nest vs. 6 meters away from the entrance.

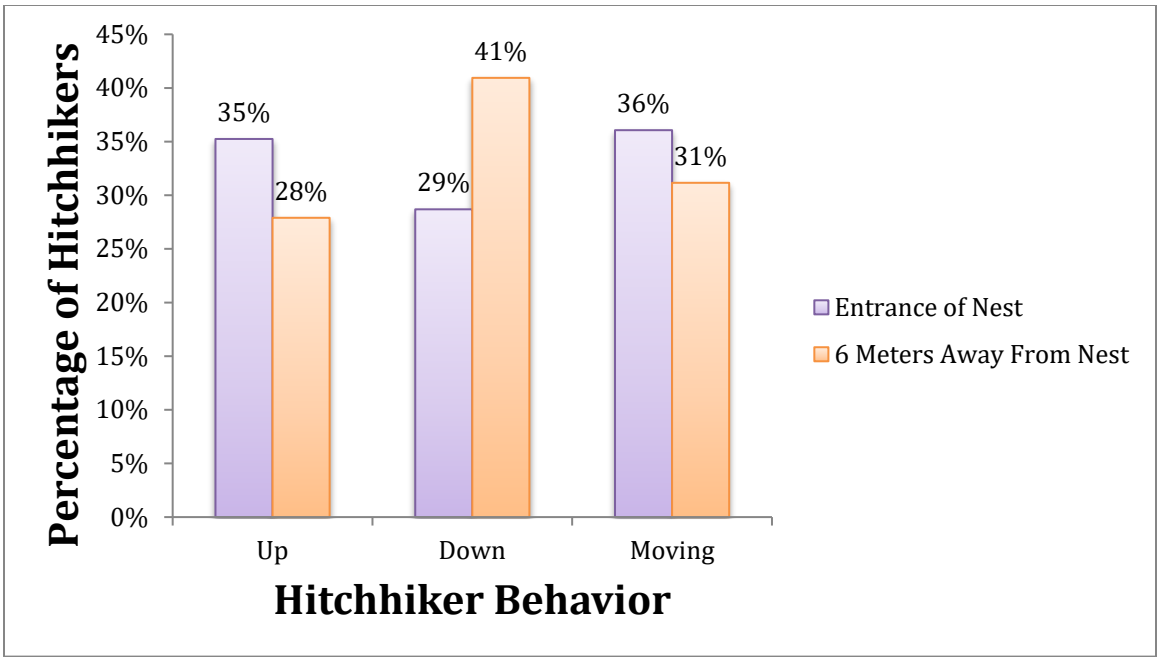

Figure 2. Hitchhiker behavior in the entrance of the nest vs. 6 meters away from the nest (%). With each behavior meaning: head up away from the leaf, head down with head facing the leaf, and hitchhiker moving around the leaf.

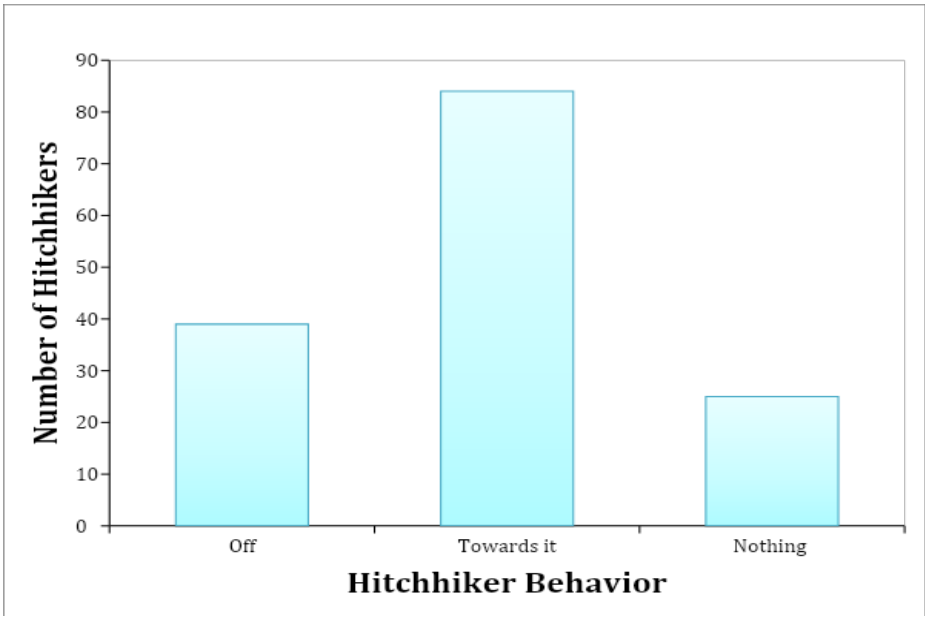

Figure 3. Hitchhiker behavior after disturbance of leaf. There is a significantly statistically difference in the amount of hitchhikers that went towards the object (x^2=38.57, $p<0.05$).

Table 1. Distance that 10 hitchhikers traveled starting at 3 meters away from the entrance of the nest.

Travel Distance	
Distance From the 3 Meter Mark (m)	Number of Hitchhikers
0-1m	5
1-2m	1
2-3m	4

DISCUSSION

Hitchhikers Protecting the Carrier Ant

The behavior of the hitchhikers (head up, head down, or moving around the leaf) can represent whether the hitchhiker is protecting the carrier ant from parasitic phorid flies by positioning it's head up. Hitchhikers were observed with their heads up but not significantly more than the other behaviors.

The hitchhikers also demonstrated a possible defense against possible parasites by going towards the object on the leaf significantly more than retreating the leaf or not reacting to it. Hitchhikers with their heads up would immediately go towards the object on the leaf and hitchhikers with their head down would either take some time to go towards the object or it would get off the leaf or continue with their head down. The observation that hitchhikers with their head down can also be protective infers that hitchhikers are aware of their surroundings. It is probable that hitchhikers protect the carrier ants from parasitic phorid flies, which supports my initial hypothesis.

The results from the hitchhiker head position and leaf disturbance, suggest that hitchhikers have more than one function throughout the trail. A study done by Linksvayer (2002) concluded that the hitchhikers have multiple functions due to the difference in the hitchhiker behavior during the day and night. In the Linksvayer (2002) study, more hitchhikers were observed to have their heads down during the night due to the inactivity of the diurnal phorid flies, and during the day the hitchhikers were mostly seen with their heads up.

Hitchhikers Cleaning the Leaf

The hitchhikers are also thought to possibly be cleaning the leaf due to the downward position of its head. On average, the hitchhikers had their heads down most of the time when it was 6 meters away from the entrance of the nest. This observation infers that if the hitchhiker is cleaning the leaf it would most likely do it when the leaf is farther away from the nest and closer to the host plant. A study done by a former CIEE student, Alex Wiltse (2016), researched the effectiveness of the hitchhikers cleaning the leaf by comparing the fungal colonies grown from leafs that had hitchhikers and leaves with no hitchhikers. The study found that leaves that had a hitchhiker grew significantly more

mold colonies than the petri dishes prepared from leaves without hitchhikers. This data shows that hitchhikers target leaves that need cleaning. The same research also used moldy flakes and starchy flakes to compare the amount of hitchhikers visited each flake. The study concluded that moldy flake had at least one hitchhiker almost five times more often than starchy flakes.

A different study performed by a former EAP student also tested the hypothesis that hitchhikers ride the leaf to clean it. This study concluded that the cleanliness of the leaf near the entrance showed no difference to leaves near the host plant. Braun (2016) also concluded that it's likely that the hitchhikers are cleaning the surface of the leaves for harmful fungi and pests. The cleaning the leaf hypothesis cannot be finalized as a fact because there are studies that both support and deny it.

Hitchhikers Eating Sap From the Leaf

The hitchhiker is also hypothesized to be eating sap from the leaf. Observing the behavior of the hitchhiker and the traveled distance by the hitchhiker tested this hypothesis. The downward head position of the hitchhiker, still or not, could mean that the hitchhiker is feeding from the sap. On average, there were more hitchhikers that were still with their head down and hitchhikers that were moving around the leaf with their head down, than hitchhikers with their head up. This behavior demonstrates that more hitchhikers could be either cleaning the leaf or feeding from the sap. In addition, the travel distance also indicates that hitchhikers either get off the leaf close to the 3 meter range from the nest or it rides the leaf the whole 3 meters into the nest. Since a small data set was observed for the hitchhiker travel distance, it will not be significant to conclude that the hitchhikers that do not ride the leaf the whole 3 meters are feeding from the sap. It will also not make sense for the hitchhikers to be adding the extra weight on the carrier ants for a long time to feed on the sap since it is not beneficial for the colony because the efficiency of the carrier ant will be diminished and take longer to carry the leaf back to the nest. The hitchhikers were also observed to get off the leaf fragment whenever the carrier ant had a difficult time going over debris left in the trail. It is probable that hitchhikers know when they are adding extra work for the carrier ant and decide to get off the leaf so that the carrier ant can reach the nest faster.

Earlier studies have actually rejected the leaf sap hypothesis because they were never actually observed feeding on the sap (Linksvayer et al., 2002), but a different study concluded otherwise. The hitchhikers showed a difference in their position between fragments of dry and fresh leaves: while in dry fragments hitchhikers were often found at the center of the fragment, in fresh fragments they were almost always at the edge of the fragment, where sap is found. This observation strongly suggests that hitchhikers do feed on the sap regardless if it does not benefit the whole colony. A study conducted by Littledyke and Cherrett (1976) also concluded that hitchhikers are drinking sap from detached leaf fragments by using radioisotopes that have shown minims ingesting leaf sap from leaves at the cutting site. Although my study found little evidence to support this hypothesis, there are previous studies that conclude that hitchhikers are eating sap off the leaf.

In the future, this study could be improved by further examining the sap feeding hypothesis by directly observing their ingestion of sap on the leaf fragments. If the hitchhikers are observed eating the sap from the leaf, then it would be compelling to also research the benefit from it since it is included in the leafcutter ant's highly organized caste system. I believe that it would be interesting to conclude that hitchhikers feed on the sap because it would infer that the sap has a significance that is also not quite known. A previous study hypothesized that since the worker ant feeds on the sap for metabolic energy, then the hitchhikers do as well (Linksvayer, 2002).

On average, more ants either had its head down or were moving around with their head down which can either mean they were feeding from the sap or cleaning the leaf. It is probable that hitchhikers are aware of its surroundings with any behavior it is performing, so it can be protective even with its head down. In addition, out of the three hypotheses, the least supported was hitchhikers feeding from the sap according to my studies. But, with other studies that support the sap hypothesis, it can be possible that the hitchhikers take advantage while they are on the leaf and feed on the sap. A combination of my study and past studies suggest that hitchhikers have multiple functions throughout the trail. These functions ensure the cultivation of the fungi by protecting the carrier ant and by cleaning the leaf. Without the hitchhikers, the colony can risk the possibility of termination due to a diminishing food source.

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