November 2010

Leaf area, worker size, and number of hitchhiking minima in Atta cephalotes

Daniel Shimek

Leaf Area, Worker Size, and Number of Hitchhiking Minima in *Atta cephalotes*

Daniel Shimek

Department of Biology, Gustavus Adolphus College

ABSTRACT

Several species of leaf-cutter ants, including *Atta cephalotes*, exhibit an interesting behavior of hitchhiking minima. Minima are the smallest of the polymorphic castes of leaf-cutter ants and the function and origin of this hitchhiking behavior is not completely understood. There are several hypotheses for this behavior, and the most popular include defense against parasites and cleaning the leaf fragment of contaminants. Studies have investigated some patterns of hitchhiking behavior including different frequencies during day and night and distance from colony. This study examined the relationship between head width, leaf area and the number of hitchhiking minima in *A. cephalotes*. Ants were collected from a single colony in Bajo del Tigre, Monteverde, Costa Rica. The number of minima, head width, and leaf fragment area were recorded. Ants with mimina present had significantly higher mean head width and carried leaf fragments with greater mean area than those without. This implies that larger ants could require more defenses from parasites or larger leaf fragments contain more contaminants.

RESUMEN

Varias especies de zompopas, incluyendo *Atta cephalotes*, exhiben un comportamiento interesante de las mínimas que viajan sobre las hojas. Mínima es la más pequeña de las castas de las zompopas y la función y el origen de este comportamiento no es completamente entendido aún. Existen varias hipótesis para este comportamiento, y la más popular incluye la defensa contra parásitos y limpiar los pedazos de hoja de contaminantes. Estudios han investigado algunos patrones de este comportamiento incluyendo diferentes frecuencias durante el día y la noche y distancia al nido. Este estudio examina la relación entre el ancho de la cabeza, área de la hoja y el número de mínimas en *A. cephalotes*. Las hormigas fueron recolectadas de un único nido en Bajo del Tigre, Monteverde, Costa Rica. El número de mínimas, ancho de la cabeza y área del fragmento cargado se anotaron. Hormigas con mínimas presentan cabezas significativamente más grandes y además también cargan trozos más grandes que aquellos sin mínimas. Esto implica que hormigas más grandes pueden necesitar más defensas contra parásitos o que los trozos más grandes contienen más contaminantes.

INTRODUCTION

Atta cephalotes is a species of leaf-cutter ant that harvests leaf fragments in order to grow a fungus for consumption. These leaf-cutter ants are important and dominant herbivores in tropical forests and can consume 12-17% of total leaf production per year (Hölldobler & Wilson 1990). Leaf-cutter ants have polymorphic castes. There are three worker castes consisting of soldiers who are the largest and defend other ants. Soldiers are found on the trails and within the nest. Media are the foragers and generalists. Media are the most common caste found on trails and perform the function of cutting and carrying leaf fragments to the nest. Minima are the smallest caste and are most commonly found within the nest caring for the fungus and larvae. However, minima are unusually and often found outside the nest along trails and often times hitchhiking on leaves carried by media foragers. This hitchhiking behavior is not very common on the trails (Feener & Moss 1989).

There have been many suggested hypotheses for the presence of minima hitchhikers. The most popular and prevalent hypothesis is that minima defended the foragers from parasitoids such as phorid flies (Phoridae) (Vieira-Neto *et al.* 2006). The flies lay eggs on the head of the ant and the larvae hatch and burrow into the head of the ant, eventually killing it. Leaf carriers can attempt to defend themselves but are rarely successful (Feener & Moss 1989). Minima have been observed in an erect head up posture apparently ready to defend the media (Linksvayer *et al.* 2002). However, phorid flies are largely diurnal in natural systems and hitchhiking behavior has been observed during nocturnal foraging (Vieira-Neto *et al.* 2006). Most minima found on trails are not hitchhiking so other explanations of hitchhiking behavior have been hypothesized. It is possible that hitchhiking may be done to conserve energy, feed on sap, clean leaf fragments or are marooned on the leaf fragment (Linksvayer *et al.* 2002). The energy conservation hypothesis states that minima that hitchhike do so to conserve energy on their way back to the nest. However the support for this hypothesis is weak at best (Feener & Moss 1989). Worker ants of *A. cephalotes* have been shown to consume a large amount of leave sap to fulfill metabolic energy requirements (Griffith &Hughes 2010). Some scientists have suggested that hitchhiking ants simply get marooned on leaf fragments and are unable to leave the fragment (Viera-Neto *et al*. 2006). This suggestion has largely been refuted in recent studies. Minima are known to remove contaminants in the fungus garden and could perform this function on leaf fragments before they enter the colony. Minima have been observed in a head down posture licking the surface leaf fragment (Linksvayer *et al.* 2002). Studies have shown increased occurrence of hitchhiking on contaminated leaf fragments and corn flakes (Vieira-Neto *et al.* 2006, Griffiths & Hughes 2010).

As the functions of hitchhiking are becoming better understood, studies have investigated further patterns of hitchhiking. One pattern that has been found is that hitchhiking occurs more commonly in the forest canopy where foraging occurs rather than close to the nest (Zisook 2006, Griffiths & Hughes 2010). However there is little information on the factors that could increase the likelihood of a minima hitchhiking such as leaf area and head size of the laden ant. Parasitic flies tend to prefer larger ants (Tonasca & Braganca 2000). Several phorid flies land on the leaf fragment in order to lay eggs in the head of the ant (Vieira-Neto *et al* 2006). One species of phorid fly (*Apocephalus attophilus*) has been shown to not parasitize ants without leaves (Feener & Moss 1989). This suggests ants with larger bodies and heads that carry larger leaf fragments would be at a greater risk from phorid flies. This is based on the assumption that a larger head correlates to ability to carry a larger leaf. These ants would therefore benefit from frequently having hitchhikers. Also, a larger leaf are could possibly contain more contaminants making it a high priority for cleaning.

These predictions formed the purpose for this experiment. The objective of this study was to examine whether a wider head or a larger leaf head would predict a higher occurrence of minima hitchhiking.

METHODS

Study Site

This study was conducted on the property of Frank Joyce near Bajo del Tigre Reserve in Monteverde, Costa Rica. The life zone of the study site is considered premontane wet forest with an elevation of ~1400 meters and mean annual rainfall of 2000-4000mm (Harber 2000).

Experimental Procedure

Five hundred and twenty eight ants were collected during the week of November 11th-18th, 2010, between 10am-2pm. I observed 264 laden ants whose leaves had zerp minima, 240 with one minima, and 23 with two minima. Ants were collected from a single colony and a single trail. The specific trail was chosen because it was observed to contain the highest density of ants. I collected all ants from the same location on the trail approximately 15m from the colony. A marker was placed along the trail and the first media which crossed the marker without minima was collected. Head width was calculated as the distance between the eyes and measured with a caliper. I measured the leaf area using a transparent grid featuring 25 mm^2 squares. I rejected ants carrying leaf stems or other leaf fragments which could not be measured with the grid. In order to collect medias with minima, the vicinity around the marker was observed and the first ant with a hitchhiker encountered was collected. I measured head width and leaf area in the same manner as ants without hitchhikers.

Statistical Analyses

I tested the mean head size by category of number of minima for statistically significant differences using a one-way ANOVA. A one-way ANOVA was used to determine differences in leaf area. A Tukey-Kramer HSD was used for post hoc comparisons between the three categories for both head size and leaf area. The correlation between head size and leaf area was tested by a bivariate linear fit.

RESULTS

Head Size

The mean head size for all ants was 0.2284915cm (±0.0431126). There was a significant difference in the mean head length between ants with zero, one, and two minima (One Way ANOVA, $F=3.4482$, $df=2$, $p=0.0325$, $R^2=0.01299$, Fig. 1). Post hoc tests revealed mean head width of ants with zero minima (0.223617cm±0.00264) was lower than ants with one minima (0.233604cm±0.00277; Tukey-Kramer HSD). Ants with two minima had a mean head width of 0.231087cm±0.00895.

Leaf Area

The mean leaf area of all ant fragments was 85.94047619 mm^2 (±31.314012). The maximum and minimum leaf areas were 250 and 18.75 mm^2 respectively. The mean leaf area between laden ants carrying leaves with zero minima, one minima, and two minima differed significantly (One Way ANOVA, $F=7.9001$, $df=2$, $p=0.0004$, $R^2=0.02927$, Fig. 2). Mean leaf area in laden ants carrying leaves with one and two minima was greater than laden ants carrying leaves with zero minima (Tukey-Kramer HSD). There was a

20% increase in mean leaf area from zero (81.274±1.9024) to two minima (102.717±6.4454). Ants with one minima showed a 10% increase in mean leaf area compared to those with zero minima (89.401±1.9953and 81.274±1.9024 respectively). Similar to head width, the R^2 value for leaf area and number of minima is quite low (0.02927) suggesting other factors might be a stronger indicator.

Head Width and Leaf Area Correlation

There was a positive correlation between head width and leaf area ($F=150.7042$, $df=1$, $p<0.0001$, $R^2=0.223033$, Fig. 3). The ant with the largest head width of 0.52cm carried a leaf fragment of 137.5 mm^2. An ant with a head width of 0.42cm carried the largest leaf fragment, with an area of 250mm^2. The smallest ant, 0.145cm head width, was found with a leaf fragment area of 37.5mm^2. The smallest leaf fragment was 18.75mm^2 and was transported by an ant with a head width of 0.16cm. Head size and leaf area were positively correlated ($R^2=0.223033$). This suggests ants with larger heads are capable or more likely carry larger leaf fragments. Also it can be inferred that ants with large heads and larger leafs together have the greatest frequency of minima hitchhikers.

DISCUSSION

A plausible explanation for why hitchhikers are likely to be found on ants with larger heads is the ant protection hypothesis that states hitchhikers defend the leaf carriers from ovipositor attacks (Feener & Moss 1989). Parasites prefer larger ants since they are more visible targets (Tonasca & Braganca 2000). Minima have been shown to successfully defend against phorid flies (Linksvayer *et al* 2002). It has been hypothesized that the presence of hitchhikers can reduce parasitism by up to 75% (Feener & Moss 1989). Media ants can attract minima through short-ranged communications including pheromones (Vieira-Neto *et al* 2006). Perhaps most hitchhiking minima only do so when summoned by a forager. This evidence strongly suggests that minima play an important role in parasitoid defense.

Hitchhikers may be found more often on larger leaf fragments for several reasons. Some species of phorid flies land on leaf fragments prior to ovipositing eggs, so larger leaf fragments might increase the chance of being parasitized (Griffiths & Hughes 2010). A larger leaf fragment might be beneficial to the fly to avoid detection or act as a landing pad or bulls-eye. This puts ants carrying a large leaf fragment at a higher risk. It is also possible that larger leaf fragments might contain more fungal contaminants because of increased area. Minima could respond by hitchhiking and cleaning these larger leaf fragments before they enter the colony and contaminate the fungus garden. Another possibility is that larger leaves could contain more leaf sap. Many of *Atta* spp. workers obtain most of their metabolic energy from sap. Perhaps larger leaf fragments contain more sap and this entices minima to hitchhike.

There are potential future studies that could address previously raised questions. To determine whether larger ants are at a higher risk, one could examine the rates of phorid parasitism and compare the mean head size of ants that become parasitized to those that do not. This could clarify whether the increased frequency of hitchhiking on large ants is a response to phorid flies. Another interesting study could focus on the contaminants on leaf fragments immediately after cutting to determine whether amount

of contaminant cover differs by size of leaf fragments. This could confirm or refute one of my speculations as to why minima are frequently found on larger leaf fragments.

Hitchhiking on leaves in *A. cephalotes* appears to be a behavioral adaptation to several selective pressures including parasite defense and fungal contaminants. This behavior seems to have evolved multiple times in different species of ants indicating a similar pattern of ovipositor behavior in their parasites.

ACKNOWLEDGEMENTS

I would like to thank my advisor Anjali Kumar for help and guidance in finding and completing this study. Moncho Calderón receives thanks for his patience and help. Raquel Martinez deserves thanks for her helpful information and insight into this project. I would finally like to thanks Pablo Allen for assistance with the statistical analyses and Frank Joyce for allowing me to use the *Atta cephalotes* colony located on his property.

LITERATURE CITED

Feener, D.H. and K.A.G. Moss. 1989. Defense against parasites by hitchhikers in leaf-cutting ants: a quantitative study. Behavioral Ecology and Sociobiology 26: 17-29.

Griffiths, H.M., and W.O.H. Hughes. 2010. Hitchhiking and the removal of microbial contaminants by the leaf-cutting ant *Atta colombica*. Ecological Entomology 35: 529-537.

Harber, W.A. 2000. Plants and Vegetation. In: Monteverde: Ecology and Conservation of a Tropical Cloud Forest, N. M. Nadkarni and N. T. Wheelwright, eds. Oxford University Press, New York, NY.

Hölldobler, B. and E.O. Wilson. 1990. The Ants. Harvard University Press, Cambridge, MA.

Linksvayer, T.A., A.C. McCall, R.M. Jensen, C.M. Marshall, J.W. Miner, and M.J. McKone. 2002. The function of hitchhiking behavior in the leaf-cutting ant *Atta cephalotes*. Biotropica 34(1): 93-100.

Tonasca, A. Jr. and M.A.L. Braganca. 2000. Forager size of leaf-cutting an *Atta sexdens* (Hymenoptera: Formicidae) in mature eucalyptus forest in Brazil. Revista de Biologia Tropical 48.

Vieira-Neto, E.H.M., F.M. Mundim, and H.L. Vasconcelos. 2006. Hitchhiking behavior in leaf-cutter ants: An experimental evaluation of three hypotheses. Insectes Sociaux 53: 326-332.

Zisook, R. 2006. Variation of hitch-hiking incidence and density in space and time in two *Atta cephalotes* colonies: effects of the presence of phorid flies. University of California, Education Abroad Program, Spring 2006.

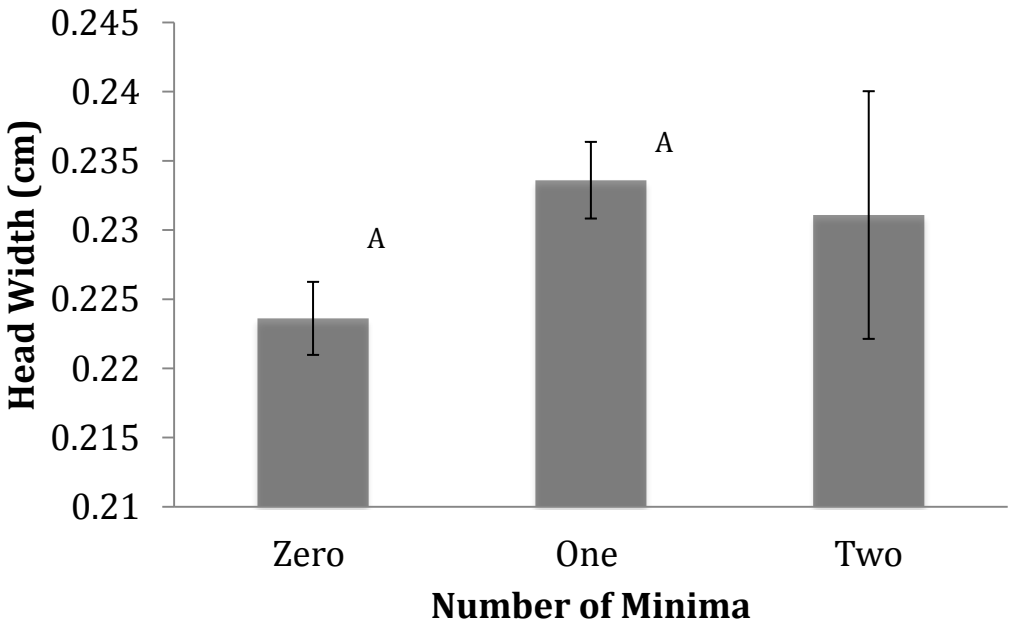

Figure 1: The mean head length of *Atta cephalotes* ants featuring three different amounts of hitchhiking minima. Similar letters indicate statistically significant difference in means (One Way ANOVA, F=3.4482, df=2, p=0.0325, R^2=0.01299). The object of this study was to determine what individual characteristics of *A. cephalotes* workers could be used to predict frequency of hitchhikers. A single colony was studied in Monteverde, Costa Rica.

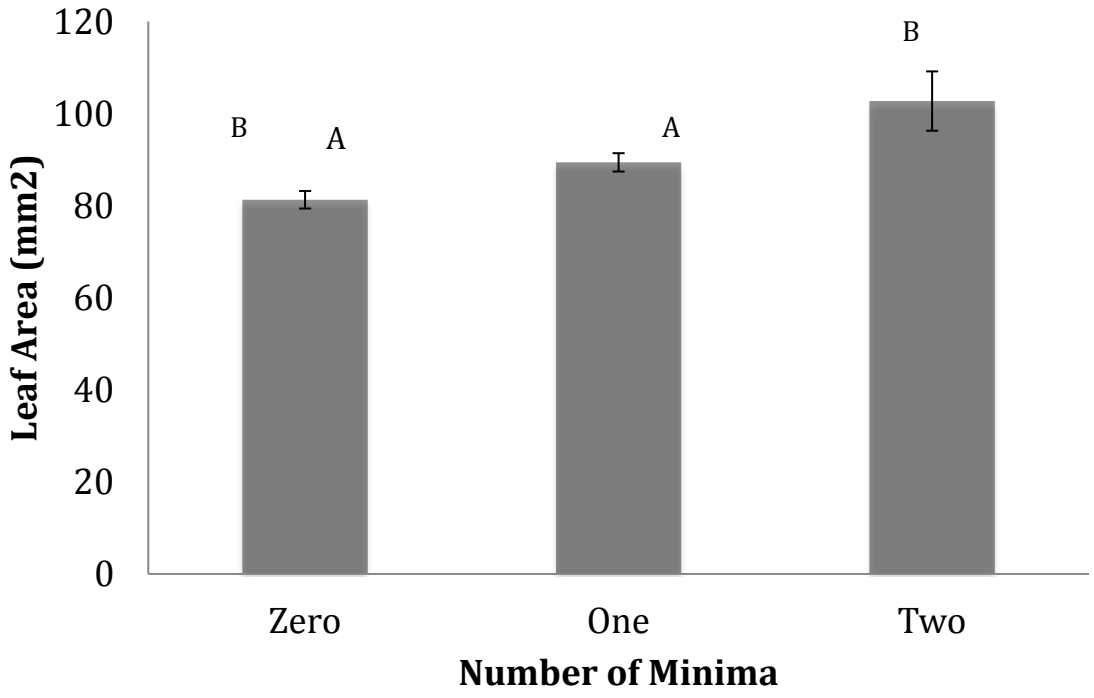

Figure 2: The mean area of leaf fragments carried by *Atta cephalotes* workers and the corresponding number of minima hitchhikers. Similar letters indicate statistically significant differences (One Way ANOVA, F=7.9001, df=2, p=0.0004, R^2=0.02927). It was hypothesized that mimina would be more frequently found on leaves with larger areas.

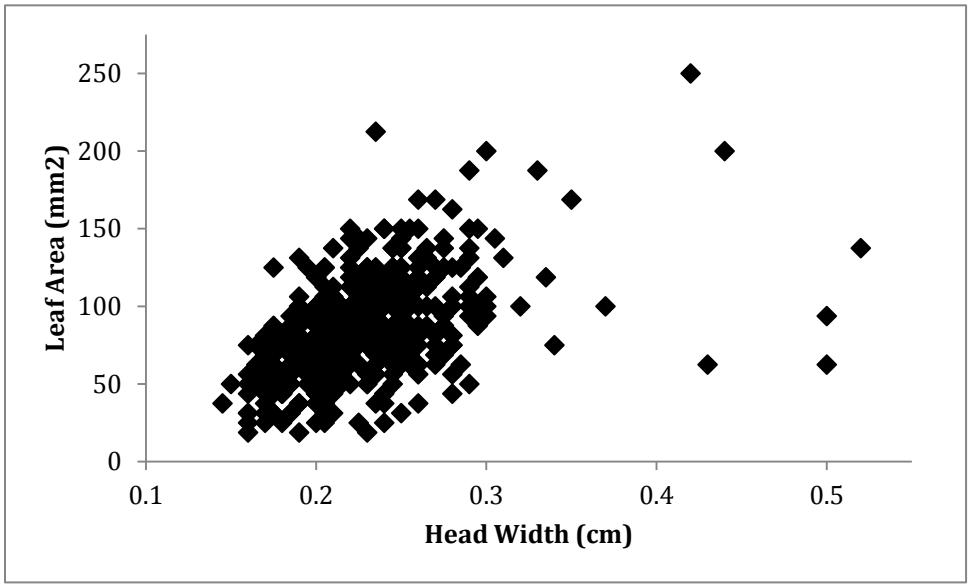

Figure 3: Correlation of head width (cm) and leaf area (mm^2) of 528 *Atta cephalotes* media workers (Bivariate linear fit, F=150.7042, df=1, p<0.0001, R^2=0.223033) It was predicted that ants with larger bodies could possibly carry larger leaf fragments or larger leaf fragments might require larger workers to carry.