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Leaf-cutter Ant (*Atta cephalotes*) Behavioral Ecology of Folivory in a Mixed-use Guyanan Lowland Rainforest

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LEAF-CUTTER ANT (*ATTA CEPHALOTES*) BEHAVIORAL ECOLOGY OF FOLIVORY IN A MIXED-USE GUYANAN LOWLAND RAINFOREST

by

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B.A., Biology, Kalamazoo College, 2012

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Abstract

I studied the foraging ecology of a Neotropical leaf-cutter ant, *Atta cephalotes*, at CEIBA Biological Center, Guyana to elucidate diet choice and foraging strategy. These ants are serous agricultural pests because workers harvest leaves, flowers, fruits, and other plant organs of both cultivated and native plants. The plant materials are used to feed symbiotic fungi whose mycelia tips are the sole food of *A. cephalotes* larvae. Leaf-cutters were usually found in human disturbed habitats especially slash-and-burned forests cleared for farms, with their higher percentage of sun-exposure and lower plant stem diameters than second growth and primary forests. When given a choice of cultivated and wild plant leaves offered in a randomized smorgasbord test, leaf-cutters accepted significantly more cultivar leaves. These had lower concentrations of secondary compounds than wild plant leaves. In addition, leaf fragment size and thickness transported by returning foragers were related to the foragers' body length, such that longer ants carried longer and thicker fragments compared to smaller ants. However, there was no relationship between travel distance to the nest and load size, recruitment and returning forager counts, or preference for cultivated plants as predicted by central place foraging theory. In summary, leaf-cutter ants at CEIBA Biological Center were found in human altered forest habitats, exhibited preferences for cultivated over wild plant organs, and did not conform to predictions of central place theory. Therefore, findings have implications for leaf-cutter ant behavioral ecology and agricultural management.

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Dedication

I dedicate this thesis to Carroll A. Jensen, my maternal grandfather who told everyone about his granddaughter studying to be a scientist just like him. Unfortunately, he died before he could see me graduate. He always reminded me to “work hard, but don’t forget to have fun!”

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Chapter 1

Impacts of leaf-cutter ants foraging in mixed-use lowland rainforest sites: a review

Elizabeth B. Karslake

> "*Leafcutters are the dominant herbivores of the Neotropics, consuming far more vegetation than any other group of animals of comparable taxonomic diversity.*"
> **In: The Ants, 1990, Hölldobler & Edward O. Wilson, Pp. 596-597**

Introduction

Leaf-cutter ants (tribe Attini, subfamily Myrmicinae) are noted fungus gardeners throughout the New World as workers harvest copious amounts of leaves including from human cultivated plants. Thus farmers in the Neotropics consider leaf-cutters as serious agricultural pests (Hölldobler & Wilson 1990; Wirth et al. 2003). Leaf-cutter ant populations increase with anthropogenic habitat disturbances i.e. from mining, farming, and human habitation as these cleared habitats provide founding queens access to burrowing grounds as well as ample pioneer plants that are favored by leaf-cutter ants (Farji-Brener 2001; Hölldobler & Wilson 1990; Leal et al. 2014). Due to increasing *Atta* spp. populations, a new approach to evaluations of the relationships between these ants and their symbiotic fungi is recommended by Leal et al. (2014). As ants feed their fungus cultivars fresh vegetation clippings, their foraging behavior is dictated by the fungi's needs (Hölldobler & Wilson 1990). Ants learn to recognize palatable plant species and avoid unacceptable plants (Saverschek et al. 2010; Wirth et al. 2003). Likewise, central place foraging optimality models can be used to predict ant foraging behavior because ants must leave from their nest, a central place, in search of fungal substrates (Burd 1996; Burd & Howard 2005; Dornhaus et al. 2006). Here I present a review of the aforementioned topics to inform my thesis research questions.

Ant-Fungus Symbiosis

Leaf-cutter ants are in a mutualistic relationship with their fungus gardens, and a recent phylogeny created from small subunit ribosomal RNA gene sequences of many fungal strains suggests that this relationship is about 50 million years old (Hinkle et al. 1994; Mueller & Gerardo 2002). While adult ants do eat the sap of leaves they cut, the main source of food for ant colonies is the fungus which workers tend by feeding small leaf fragments and removing any competing fungi (Hölldobler & Wilson 1994). Success of leaf-cutter ant colonies depends on the intensity of care of fungus gardens by the ants that effectively manage fungal diseases (Mueller & Gerardo 2002). Some of that control is exerted by *Streptomyces* spp. bacteria which live on the bodies of leaf-cutter ants as well as in the colony that help protect against invasions from antagonistic fungi, such as *Escovopsis* spp. which can attack the ants' cultivated fungi (Mueller & Gerardo 2002). Therefore the cultivars, with most fungal species having co-evolved with a particular ant species, are dependent upon ants for survival, and have never been found outside of ant colonies (Chapela et al. 1994; Hinkle et al. 1994). The species of fungus tended by *Atta cephalotes* is similar to the fungus of *Trachymyrmex* and *Sericomyrmex* but is larger to sustain *A. cephalotes* colonies which can contain several hundred thousand individuals (Hölldobler & Wilson 1990; Hinkle et al. 1994).

Interactions between leaf-cutter ants and their fungi include two proposed mechanisms that prevent foreign fungal strains from invading ant colonies and stabilized coevolution between ants and their cultivars (Mueller & Gerardo 2002). The first mechanism described by Mueller et al. (2004) allows horizontal transmission of fungus from one colony to another after a colony's previous strain goes extinct due to infections from pathogens. The second mechanism is the partnership feedback already existing between established fungus and its ant colony. In this partnership there is only vertical transmission of cultivar from one colony to another (Mueller et al. 2004; Seal 2006). This mutualism could have first come about through ants foraging on fungi or through facilitation as fungi colonized a refuse dump near an ant colony. Either way, a very effective mutualism has developed (Mueller & Gerardo 2002).

During horizontal transmission of a fungus strain from one colony to another, ants distinguish between superior and inferior strains of fungi (Mueller et al. 2004). A superior strain is defined as a cultivar more closely related to a colony's previous fungus compared to a more distantly related strain. Mueller et al. (2004) tested the ability of *Crphomymex meulleri* ants to distinguish among cultivar strains and decide which would be more compatible. To determine *C. meulleri* symbiont choice, worker ants were exposed to representative fungal species from the clade containing their own cultivar. Ants chose the strain most closely related to their native cultivar (Mueller et al. 2004). It was concluded that if a colony's garden became depleted ants are capable of choosing superior replacement strains (Mueller et al. 2004). Therefore, co-evolution between leaf-cutter ants and fungus strains stabilizes the mutualism through the ability of leaf-cutters to distinguish among strains and fungi-ant partnership feedback (Mueller et al. 2004).

The partnership between a leaf-cutter ant colony and its cultivar is maintained by the ants' ability to attend to cues from their fungus (Hölldobler & Wilson 1994). Foraging behavior of *Trachymyrmex seplentrionalis,* a North American fungus gardening ant, is influenced by fungus growth rate (Seal 2006). If there is a decrease in growth rate of the cultivar, ants respond by rejecting food items associated with garden decline and place these in refuse piles outside of the nest. Ants then bring in different mulching items, and if items are acceptable for fungus growth ants are rewarded with positive feedback (Seal 2006). For example, laboratory colonies of *T. seplentrionalis* initially collected mostly frass (caterpillar feces), but later switched to collecting fragments of oak tree bark. These ant colonies were rewarded with positive feedback as fungus gardens grew quickly (Seal 2006). After fungus gardens have grown to a large size and workers are no longer hungry, ants respond to this negative feedback by collecting fewer plant materials (Burd & Howard 2005; Seal 2006). Foraging activity resumes when workers are sufficiently hungry. Therefore, Seal (2006, pg. 20) concluded that for "correct choices to emerge and colony performance to be optimized, workers must interact with both their nest-mates and fungus gardens."

Division of labor in leaf-cutter ant nests optimizes colony performance (Hölldobler & Wilson 1994; Wirth et al. 2003). Larger ants cut up leaves into manageable fragments and transport these to the nest where they are dropped onto the colony floor in a pile. Smaller ants clip leaf fragments to about 1 mm wide discs, and these are chewed into a pulpy mass by even smaller ants (Hölldobler & Wilson 1994). This substrate is molded into small pellets and inserted into fungus gardens. Ants provide some of the necessary digestive enzymes and amino acids to assist fungus digestion of leaves (Martin 1970). Growing cultivar inserts its hyphae into the substrate and spreads along the ridges like a bread mold (Hölldobler & Wilson 1994). The smallest ants patrol the cultivar gardens, navigating through narrow channels, and constantly touch the cultivar with their antennae. They remove spores and hyphae of alien fungi as well as pluck ripe cultivar hyphae to feed their nest-mates (Hölldobler & Wilson 1994). Soldiers patrol among foraging ants and aid in the defense of the colony. Typically tasks are specified by size, but in smaller colonies individuals are more likely to multi-task (Hölldobler & Wilson 1994). However, there is thought to be communication between ants that remain in the nest and foraging ants about the palatability or unpalatability of leaves incorporated into fungus substrates thereby preserving a colony's ant-fungus symbiosis (Wirth et al. 2003).

Foraging Behavior

Maintenance of foraging trails by scouts and recruited foragers allows leaf-cutter ants access to acceptable leaves. Trails are marked chemically with pheromones having two odor cues, and pheromones are secreted from ants' poison gland sacs (Hölldobler & Wilson 1990; Wirth et al. 2003). Workers constantly communicate through stridulating (production of sound by rubbing the gastor or mandibles while cutting leaves) about quality and locations of leaves currently being harvested (Wirth et al. 2003). Returning scouts may also recruit nest-mates to host plants through leaf odors (Roces 1990). One function of pheromones is to serve as an orientation cue to help foragers locate chosen vegetation patches (Wirth et al. 2003). Interestingly, locations with acceptable leaves are specified by marking both the trunk and branches of plants (Wirth et al. 2003). Long distance foraging routs are repeatedly re-marked by ants traveling along these trails (Jaffé & Howse 1979), and higher pheromone concentrations indicate higher quality of foraging patches (Jaffé & Howse 1979; Wirth et al. 2003). Thus, the higher the pheromone concentration the more workers are recruited to harvest leaves (Jaffé & Howse 1979).

Silva et al. (2013) examined several forest foraging trail attributes to elucidate the spatiotemporal architecture of *A. cephalotes* foraging trails over a 12 month period. They mapped the foraging system of ant colonies in Atlantic forest patches and trail system attributes. The trail architecture varied with patch size, regeneration age of already defoliated patches, and abundance of pioneer plants (new growth plants; Silva et al. 2013). Trail complexity was not correlated with patch size and age, but was positively correlated with the number of pioneer plants and trees across secondary forest patches. Trail complexity varied throughout the 12 month period but overall trail abundance increased with the abundance of pioneer plant stems (Silva et al. 2013). This allows ants to fine tune trail networks and profit from harvesting newly emerging leaves of previously known plants as well as newly discovered hosts (Silva et al. 2013). Factors effecting leaf quality include leaf nutrients, moisture content, salt concentration, the presence of endophytic fungi, and leaf toughness (Chavarria Pizarro et al. 2012; Coblentz & Van Bael 2013; Howard 1996; Nichols-Orians & Schultz 1989). Thus, *A. cephalotes* maintain highly flexible trail networks by fine-tuning their foraging trails to gain access to the highest quality leaves

Plant Species Preferences

Leaf-cutter ants are acutely aware of the nutritional needs of their fungal cultivars and selectively forage for substances beneficial to their fungi (Hölldobler & Wilson 1990). Leaf-cutter ants usually prefer leaves with low levels of secondary metabolic compounds that are produced by plants to deter herbivores (Howard 1987). Of 42 randomly sampled plant species from a dry forest in Costa Rica, 75% contained significant numbers of repellent non-polar extractables or volatiles such as terpenoids, steroids, and waxes, and 50% of the species contained significant repellent polar extractables such as phenols, flavonoids, and glycosides (Hubbell et al. 1984). Occurrences of these non-polar volatiles correlate positively with plant species avoided by free-living *A. cephalotes* (Hubbell et al. 1984). Isolation of ant repellent extractables showed many to be terpenoids which are highly toxic to the fungal cultivar (Hubbell et al. 1984). In an earlier study by Hubbell et al. (1983), *A. cephalotes* avoided leaves of *Hymenaea courbail* because of its terpenoid compounds. Later, when this terpenoid—caryophyllene epoxide—was extracted and added to palatable leaves the ants rejected these previously accepted samples (Hubbell et al. 1983).

Leaf-quality is also affected by environmental conditions. One study tested the hypothesis that leaf quality variation within a plant species is partially due to environmental differences inducing changes in secondary chemicals like tannins (Nichols-Orians 1991). To test this hypothesis *Inga osretediana* seedlings were raised under varying environmental conditions. Leaves of this species are typically palatable to leaf-cutter ant fungi cultivar gardens, and naturally grow in areas with just 1-2% light penetration under the dense tree covers of forests or in clear areas with 20% light. Seedlings were raised in environments with both of these light percentages and varying fertilization levels (Nichols-Orians 1991). Leaves that grew to maturity under different soil, light, and fertilization treatments did differ in tannins concentrations, and plant growth effected tannins concentration. However, once a leaf matured, tannins concentrations did not change as a function of either light or soil nutrient conditions, and levels of tannins were not affected by growth rate. As expected, leaves that expanded to maturity under different conditions differed in levels of acceptability to ants, but decreased levels in concentrations of tannins did not result in mirrored increase acceptability (Nichols-Orians 1991). Foraging *A. cephalotes* ants preferred leaves of seedlings grown at 20% light, even though these had higher tannins concentration, over seedlings grown at 2% light. Acceptability of seedling leaves grown at 2% light with higher levels of fertilization were preferred, because these had lower levels of tannins, over seedling leaves grown under similar conditions but with lower levels of fertilization (Nichols-Orians 1991). Nichols-Orians (1991) concluded that spatial variation in resource availability for plants, as potentially experienced in the lowland tropical forests, can result in differences of tannin chemistry and leaf acceptability to ants.

While water and sucrose are needed by leaf-cutter ants, higher salt concentrations seem to increase the quality of leaves (Chavarria Pizarro et al. 2012). Salt is a limiting nutrient for herbivores including leaf-cutter ants. To test the hypothesis that leaf-cutter ant colonies are sodium limited Chavarria Pizarro et al. (2012) offered bits of paper soaked in either $C_{12}H_{22}O_{12}$ (sucrose), NaCl, Na_2SO_4, KCl, or water. While foraging *A. cephalotes* mostly accepted pieces of sucrose soaked paper, ants did take pieces of paper containing Na^+ ions more often than bits of paper soaked in water or KCl (Chavarria Pizarro et al. 2012). Since, there was no significant difference between acceptance of paper soaked in either NaCl or Na_2SO_4 the authors concluded that ants preferably foraged for Na^+ ions over anions such as Cl^-. Therefore the hypothesis that leaf-cutter ant colonies are in demand for sodium and that foraging behavior of ants is dictated by this need was supported (Chavarria Pizarro et al. 2012).

Ants select leaves containing smaller populations of endophytic fungi that could compete with their cultivar gardens (Coblentz & Van Bael. 2013). In Panama, *A. colombica* foragers returned with leaf clippings, flower pieces, and fragments of fruit, because these usually have 20% fewer endophytic fungi compared to conspecific leaves (Coblentz & Van Bael 2013). Using leaf clippings obtained from foraging ants, Coblentz & Van Bael (2013) created a model describing leaf endophytic fungi concentration of plants around colonies to predict what types of leaves ants would select (Coblentz & Van Bael 2013). By collecting leaves with lower levels of endophytic fungi ants decrease the amount of competing fungi entering the nest by 33%. This supports previous findings of negative interactions between fungus gardens and endophytic fungi, leading Coblentz & Van Bael (2013) to conclude that selective foraging by leaf-cutters plays a defensive role of protecting fungus gardens.

Preference for young leaves could have come about because these leaves have lower levels of endophytic fungi, are tender, and are easier to cut, and sometimes have fewer plant volatiles (Silva et al. 2013; Wirth et al. 2003). In fact, Nichols-Orians & Schultz (1989) found that *A. cephalotes* foragers harvest more young leaves than old leaves of an unidentified Rubiaceous tree. They tested the hypothesis that workers prefer to cut tender leaves by presenting foraging ants with Rubiaceous tree leaves. Nichols-Orians & Schultz (1989) found that while there were no significant preferences for either young or old leaf disks, most workers seemingly preferred tender leaves (Nichols-Orians & Schultz 1989). Only larger ants with larger head capsules were capable of cutting up older, tougher leaves, and these occurred at much lower frequencies (Nichols-Orians & Schultz 1989). Tougher leaves had longer cutting times than tender leaves, and this may explain why the majority of leaf-cutter ants preferred more tender leaves with lower levels of endophytic fungi (Nichols-Orians & Schultz 1989).

Foraging Experiences and Preferences

Foraging preference is determined by ants' past foraging experience as well as leaf quality (Howard et al. 1996). Howard et al. (1996) used six *A. colombica* colonies and presented them with experimental vegetation patches containing two species, *Aphelandra golfodulcensis* and *Caryocar costaricense*. These plants varied in acceptability to the ants, and the foraging behavior of scouts and recruited workers was observed and timed (Howard et al. 1996). A plant species was considered familiar when occurring in the foraging range of the ant colony such that *A. golfodulcensis* was close to two colonies and *C. costaricense* near to two others. Two other ant colonies did not encounter *A. golfodulcensis* or *C. costaricense* but foraged on fallen fig fruits. Scouts exhibited faster recruitment behavior after encountering a patch containing familiar plants (Howard et al. 1996). However, when a scout returned from a mixed patch recruited ants harvested any plant species, including *A. golfodulcensis* or *C. costaricense,* on the foraging trail regardless of the species carried home by the scout. While naïve ant colonies accepted both *C. costaricense* and *A. golfodulcensis* there was delayed rejection of *A. golfodulcensis* after 24 h (Howard et al. 1996). Colonies in areas where *A. golfodulcensis* occurred continued to accept this species even after two days of exposure to *C. costaricense*, but colonies where *C. costaricense* occurred continually accepted both. Howard et al. (1996) concluded that conditioning affects relative acceptability of resources to both scouts and recruits, and this could partially explain the variance in resource preference among ant colonies.

Furthermore, *A. colombica* exhibited delayed rejection (i.e., discarded plant species after initial acceptance into the nest), and avoidance of 10 rarely harvested plant species (Saverschek et al. 2010). In this study, 10 leaf-cutter ant colonies in Panama were given the opportunity to choose among 7 mm disks punched from leaves of rarely harvested plants. Ants from colonies where the study plants naturally occurred avoided all of these samples upon initial encounter suggesting previous experience with the plants (Saverschek et al. 2010). However, colonies where the plants did not occur initially accepted the leaf disks. Later, the ants demonstrated delayed rejection 24 and 48 h after samples were first introduced. Then to test how robust learning and memory of unpalatable plants was, previous acceptable leaves were inundated with a fungicide (cycloheximide) that is undetectable by the ants, and foraging decisions of workers were observed for several months. After the first couple of days leaf-cutter ants learned to avoid this plant and it was 18 weeks before workers harvested it again. This indicated learning of long term avoidance (Saverschek et al. 2010). Similar observations of delayed rejection and long-term avoidance of leaf samples containing fungicide were demonstrated by *Acromymex lundi* (Herz et al. 2008). This flexible change in foraging behavior may be a mechanism to avoid injuring the fungus garden through contamination by harmful compounds in unpalatable substrates (Herz et al. 2008). Saverschek et al. (2010) concluded that harvesting and avoidance behavior of ants was dependent on workers' foraging experience and this adaptation was essential in an environment where leaf availability and quality varied throughout the year. Thus, leaf-cutter ant foraging preference is influenced by many factors.

In summary, leaf-quality is defined by the quantities of nutrients and plant metabolites they contain (Howard 1987). Leaves with more water and sugars are needed by the food fungi of the ants, but volatiles and endophytic fungi can be harmful (Howard 1987). Likewise, salt and other supplements are needed by the colony (Chavarria Pizarro et al. 2012). Younger leaves are easier for most workers to cut (Wirth et al. 2003). Intimate interactions among worker ants and fungi help communicate information of what leaf types are most palatable to gardens (Seal 2006; Wirth et al. 2003). However, foraging experience and by extension preferences are limited to what plants are available to the ants for fungal diets (Howard et al. 1996). If ants encounter new vegetation types they can learn to recognize and avoid unpalatable types through delayed rejection (Saverschek et al. 2010). Recognition and learning of acceptable and unpalatable leaf types optimizes leaf-cutter ant foraging behavior (Roces 1990; Saverschek et al. 2010). Therefore, optimality models can be used to predict optimal foraging behaviors by Attini ants (Kacelnick & Cuthill 1990).

Optimality Models

From the mid-1960s to the early 1990s there was a proliferation of mathematical and graphical models that make quantitative predictions about simple decision making processes and optimal foraging strategies by all animals (Dornhaus et al. 2006; Kacelnik & Cuthill 1990; Orians 1980; Schoener 1979; Stephens & Krebs 1986). Predictions can be used to determine whether the stated hypothesis is realistically represented by the model (Orians 1980). Foraging behavior requires animals to make decisions such as where to forage, what prey items to select, and when to move to another more profitable patch (Dornhaus et al. 2006; Olsson et al. 2008). Decisions are guided by cost/benefit ratios (McNamara 1982; Olsson et al. 2008). Animals should seek prey items with the most energetic net gain to optimize their benefit, but there are costs when returning to the nest with heavy loads that expend more energy than necessary (Orians 1980). However, animals living under variable natural conditions have an incomplete knowledge about their environment and may not be naturally selected to make decisions that minimize all costs(McNamara 1982). Optimal foraging models can be designed to accommodate this, as individuals then must make foraging decisions based on their limited knowledge (McNamara 1982).

In a changing environment, animals, such as leaf-cutter ants, benefit by retaining information learned from previous experiences (Dunlap & Stephens 2012). Through tracking environmental changes, an animal learns to choose the best strategy and optimize decisions. Dunlap & Stephens (2012) found that old information must be balanced with recently learned information by testing memory retention in blue jays (*Cyanocitta cristata*) in response to a changing environment. They posited that memory retention length should be tied to ecological relevance and the value of information learned, and that environmental change is a main determinate of the value of a memory. Under laboratory conditions, blue jays encountered environmental changes at either high or low rates as generated by computer simulation (Dunlap & Stephens 2012). A bird's ability to respond using information gained within the last hour (recent) or several days previously was measured to determine whether they integrated past and more recent information. Dunlap & Stephens (2012) predictions were confirmed because the jays sampled more often and learned more quickly when the environment changed frequently. Interestingly, blue jays showed a balanced retention of both previous and recently acquired information when environmental change occurred at a higher rate (Dunlap & Stephens 2012). This mechanism could possibly explain how leaf-cutter ants learn to recognize and avoid unpalatable plants for extended periods of time (Saverschek et al. 2010).

Central Place Foraging Applied to Attini Ants

The appropriate optimization model to assess leaf-cutter foraging decisions is central place foraging theory (CPF; Burd & Howard 2005; Orians 1980). CPF theory has been used to model foraging of nesting birds, hymenoptera, wood rats (*Neotoma floridana*), and human hunter-gather human populations (*Homo sp.*) that are obligated to leave from a central place in search of food or other items (Dornhaus et al. 2006; Kacelnik & Cuthill 1990; McGinley 1984; Starkovich 2015; Orians 1980). Attini ants are also central place foragers as they must leave their nest to encounter plant species at various travel distances from the nest (Burd 1996; Burd & Howard 2005; Dornhaus et al. 2006). Foragers are predicted to select loads that optimize their performance and reduce energetic costs as well as forage at patches that maximize energetic intake (Dornhaus et al. 2006; Olsson et al. 2008; Olsson & Bolin 2014; Orians 1980). There is a proposed tradeoff between travel distance from the central place and acceptance of patch types (Olsson & Bolin 2014). Individuals who travel farther away should select to forage at higher quality sites, but animals that stay closer to the nest may forage at both higher and lower quality patches (Olsson & Bolin 2014). Therefore, CPF theory expects foraging decisions to be affected by 1) travel distances from a central place to foraging patches, 2) handling cost of each load, 3) recruitment rate for social foragers (such as leaf-cutter ants), and 4) quality of items to be harvested (Orians 1980; Dornhaus et al. 2006; Olsson & Bolin 2014). In summary, for leaf-cutter ants, CPF models can be used to make predictions about recruitment rate, load size, returning velocity, and selection of vegetation patches depending on distance and quality (Burd 1996; Burd & Howard 2005; Dornhaus et al. 2006; Kacelnik 1993; Roces & Nunez 1993).

The mass dependent costs hypothesis predicts lighter loads will be favored by returning foragers (Dornhaus et al. 2006; Kacelnik 1993). For example, returning *A. colombica* workers often carry loads well below the mass needed to maximize energetic gains predicted by CPF theory (Burd 1996; Burd & Howard 2005). There are also documented correlations among leaf-cutter ant head, mandible, and femur length with load mass such that larger ants can carry heavier loads (Burd 1996; Wirth et al. 2003). However, leaf load size may restrict ants of all sizes when carrying heavier loads up vertical slopes as this can be very strenuous (Wirth et al. 2003). Likewise in honeybees, individuals expend more energy traveling from flower to flower with an increasingly heavy and full load than retuning with a partially full load (Dornhaus et al. 2006). If foragers expend more energy returning with a full load than gained, the net energy gain is negative. Thus, CPF models can be designed so that lighter loads are favored (Dornhaus et al. 2006).

The information transfer hypothesis predicts sub-maximial load delivery by leaf-cutter ants (and honeybees) as this facilitates information exchange at the nest (Dornhaus et al. 2006; Kacelnik 1993; Roces & Nunez 1993). Information exchange starts with workers returning to the nest carrying light leaf loads from a high quality site, and Roces and Nunez (1993) describe expected behavior of recruited leaf-cutter ants (*Acromyrmex lundi)*. One scout was allowed to feed on a sucrose rich solution and return to the nest displaying recruitment behavior (tapping its abdomen to the ground). In experiment 1, a piece of parafilm soaked, either in 1% or 10% sucrose solutions were replacements for the sucrose solution and recruited foragers harvested this. It was observe that ants cut smaller fragments when harvesting 10% sucrose soaked parafilm and returned to the nest more quickly. Likewise, when harvesting from 1% sucrose soaked parafilm ants cut larger and heavier pieces and returned to the nest with slower velocity (Roces & Nunez 1993). In experiment 2 ants were offered filter paper soaked in either 1% or 10% sucrose. This controlled for fragment size and mass because filter papers had the same mass and foragers could easily pick up one pieces of filter paper. Again ants returned more quickly with 10% sucrose soaked filter paper than with 1% sucrose soaked filter paper. Therefore, when returning from a higher quality site ants should cut lighter loads, run with higher velocity (Kacelnik 1993). Likewise, scouts and foragers are more likely to display recruitment behavior when returning from a high quality foraging patch, and foragers continually mark and maintain foraging trails leading to good quality vegetation patches (Jaffé & Howse 1979; Wirth et al. 2003).

Interactions among workers are expected to communicate the need for certain fungus-substrate types, smaller loads, and slower transfer rate of substrate fragments to reduce congestion of leaf-fragments in nest tunnels (Burd 1996; Burd & Howard 2005; Wirth et al. 2003). Burd & Howard (2005) posited that the underground processing of leaf fragments restricts the overall delivery rate of ants above ground, generating the prediction that smaller leaf loads are favored over larger ones. Researchers observed three captive colonies as workers delivered pre-cut leaf fragments of various sizes and shapes to the nests. Leaves were then moved sequentially from chamber to chamber until they reached the fungus gardens where they were trimmed into slender strips and integrated into the fungus (Burd & Howard 2005). Leaf fragment size had strong effects on all processes (tissue transfer between chambers, hoisting, cleaning, and shredding of leaf fragments), except hoisting. Overall, smaller leaf fragments were processed quicker than larger ones. The time needed to complete underground activities was longer than time needed to deliver the leaves. Although CPF theory predicts that workers carry larger loads than observed to minimize energetic costs of foraging, Burd & Howard (2005) concluded that load selection by foragers may have evolved to optimize the processing of leaves at the fungus garden. Burd & Howard's (2005) study was not a true test of CPF theory because they fitted data to CPF theory in a *post-hoc* manner and did not explicitly test CPF predictions. However, Burd & Howard (2005) still offer a plausible explanation for why smaller leaf-fragments might be favored by leaf-cutter ants (Dornhaus et al. 2006).

The aim of Dornhaus et al. (2006) was to unify these three explanations, mentioned above, and develop a model that explained deviations from load size maximization. Dornhaus et al. (2006) predicts that information about food source locations can be crucial to decision making processes of organisms of where to forage, how soon to move to a new foraging site, and partial load size. In particular the exchange of information concerning sites of high quality patches will facilitate sacrifices of workers returning from a lower quality site as they return faster to the central place carrying smaller loads and leave sooner for higher quality sites (Dornhaus et al. 2006). In the model there was the possibility of foragers returning from one food source to learn about locations of higher quality source at the nest. In these cases foragers are expected to return early from food sources with partial loads. While the forager is not achieving maximum food delivery rate, there may be maximization at the colony level. Results indicate that returning with partial loads by the individual does not always ensure that it will learn about a higher quality site. However, collected data from honeybees and leaf-cutters fits the hypothesis that returning foragers purposely reduce loads to give nestmates information about good quality foraging patches. Results support the hypothesis that there is maximization at the colony level rather than the individual level (Dornhaus et al. 2006). Efficient distribution of information by successful foragers may be necessary in successful foraging by social honeybee and leaf-cutter ant colonies (Dornhaus et al. 2006).

Therefore, there is support for the information transfer hypothesis and interaction among workers explanation (Dornhaus et al. 2006). However, Dornhaus et al. (2006) suggested that several hypotheses should be used to explain optimal foraging strategies by leaf-cutter ants and other hymenoptera. Kacelnick (1993), however, concluded that of three hypotheses based on CPF theory as applied to leaf-cutter ants the information transfer hypotheses had the most support. Roces and Nunez (1993) is one of the few, if only, studies that has shown what foraging behaviors to expect under the information transfer hypothesis. Leaf-cutter ants can sometimes carry lighter loads than expected Burd (1996), and Burd & Howard (2005) propose that selection of smaller leaf fragments by leaf-cutter ants evolved because leaf processing in the nest is slower than the gathering of fungus-substrates above ground.

Preliminary Unpublished Findings and Project Direction

Atta cephalotes at Karanambu in Guyana demonstrate a preference for cultivated plants over forest species (Burton & Holden 2012). As mentioned above ants generally prefer leaves with low levels of secondary compounds (i.e., non-polar volatiles; Hölldobler & Wilson 1990; Howard 1987). *Atta cephalotes* readily abandon forest plant leaves for leaves of human cultivars, and the rank leaf preference order with increasing handling times was cassava (*Manihot esculenta*), orange (*Citrus sinensis*), avocado (*Persea Americana*), lemon (*C. limon*), lime (*C. aurantifolia*), grapefruit (*C. paradise*), mango (*Mangifera indica*), and jamoon (*Syzygium cumini*; Burton & Holden 2012). In a reciprocal study also at Karanambu in January 2014, ants did not abandon cultivated leaves they were transporting for native plant species (Perks & Moore 2014). The conclusions from these aforementioned studies and others are that *A. cephalotes* prefer crop leaves over forest plants because human cultivars were selected to have lower quantities of plant defensive compounds such as plant metabolites (Burton & Holden 2012; Wirth et al. 2003; Perks & Moore 2014). However, more studies are needed to understand how changes in herbivore resistance traits, due to domestication, affect interactions across multiple trophic levels (Chen et al.2015).

In a pilot study at CEIBA Biological Center I investigated the question why do *A. cephalotes* demonstrate a preference for crop leaves when surrounding forests have more available leaves (Karslake 2014)? By presenting ants, already transporting leaves to their nests with a smorgasbord of forest and crop leaf samples, I observed a slight preference for crop leaves as more crop leaves were accepted over non-crop leaves. Although, there were no significant differences for discovery or handling times of forest and crop leaf species, the observed preferences for crop leaves may be due to lower levels of defensive compounds harmful to the ant's cultivar (Karslake 2014). However, significant preference differences among colonies were measured (Karslake 2014). Likewise, when I offered ants a randomized smorgasbord solely containing crop leaves common around CEIBA there was again preference variation among colonies. As mentioned above, while fungi can detoxify some harmful secondary compounds, ants should select leaves with lower levels of defensive chemicals (Howard et al. 1996). Therefore, ants should favor leaves based on leaf quality and previous experience (Saverschek et al. 2010). Ants may accept leaves initially that are previously not encountered, but if leaves are unpalatable ants are expected to display delayed rejection (Wirth et al. 2003).

Given the findings of this review, several problems remain to be solved. Leaf-cutter ants are a tractable model organism—easy to observe, and with a rich natural history literature (Hölldobler & Wilson 1990). Although, quite a few facts are already known about their foraging behavior and leaf preferences, ecological theory applied to leaf-cutter ants is now ripe for *a priori* testing (Burd & Howard 2005; Wirth et al. 2003). My thesis is designed to achieve three aims: 1) identify ecological variables associated with leaf-cutter ant habitat occupancy and ant absence. This will be the subject of Chapter 2 of my thesis; 2) test the hypothesis that *A. cephalotes* prefer crop leaves to forest leaves because many crops lack volatiles found in native species that repel leaf-cutter ants (Chapter 3); and 3) test the hypothesis that foraging behavior of *A. cephalotes* confirms to CPF theory predictions (Chapter 4). My thesis will add to the growing leaf-cutter ant literature by more accurately describing habitats occupied by leaf-cutter ants, and what types of leaves leaf-cutter ants consider higher quality (human cultivated or native species).

Literature Cited

BURD, M. 1996. Foraging Performance by *Atta colombica,* a leaf-cutting ant. *The American Naturalist* 148: 597-612.

BURD, M. & J. J. HOWARD. 2005. Central-place foraging continues beyond the nest entrance: the underground performance of leaf-cutting ants. *Animal Behaviour* 70: 737–744.

BURTON, B. & F. HOLDEN. 2012. Smorgasbord trials of leaf species preferences by the leaf-cutter ant, *Atta cephalotes*. Abstracts of URS, University of Missouri-St. Louis, St. Louis.

CHAPELA, I. H., R. A. REHNER, T. R. SCHULTZ & U. G. MUELLER. 1994. Evolutionary history of the symbiosis between fungus growing ants and their fungi. *Science* 266: 1691-1694.

CHAVARRIA PIZARRO, L., H. F. MCCREERY, S. P. LAWSON, M. E. WINSTON & S. O'DONNELL. 2012. Sodium-specific foraging by leafcutter ant workers (*Atta cephalotes*, Hymenoptera: Formicidae). *Ecological Entomology* 37: 435-438.

CHEN, Y. H., R. GOLS & B. BENREY. 2015. Crop domestication and its impact on naturally selected trophic interactions. *Annual Review of Entomology* 7: 35-58.

CLARK, C. W. 1994. Leaf-cutting ants may be optimal foragers. *Trends in Ecology and Evolution* 9: 63.

COBLENTZ, K. E. & S. A. VAN BAEL. 2013. Field colonies of leaf-cutting ants select plant materials containing low abundances of endophytic fungi. *Ecosphere* 4: 1-10.

DORNHAUS, A., E. J. COLLINS, F.-X. DECHAUME-MONCHARMONT, A. I. HOUSTON, N. R. FRANKS & J. M. MCNAMARA. 2006. Paying for information: partial loads in central place foragers. *Behavioral Ecology and Sociobiology* 61:151–161.

DUNLAP, A. S. & D. W. STEPHENS. 2012. Tracking a changing environment: Optimal sampling, adaptive memory and overnight effects. *Behavioural Processes* 89: 86–94.

FARJI-BRENER, A. G. 2001. Why are leaf-cutting ants more common in early secondary forests than in old-growth tropical forests? An evaluation of the palatable forage hypothesis. *OIKOS* 92: 169–177.

HERZ, H., B. HÖLLDOBLER & F. ROCES. 2008. Delayed rejection in a leaf-cutting ant after foraging on plants unsuitable for the symbiotic fungus. *Behavioural Ecology: E1-E8* Advance Access published February 28, 2008.

HINKLE, G., J. K. WETTERER, T. R. SCHULTZ & M. L. SOGIN. 1994. Phylogeny of the attine ant fungi based on small subunit ribosomal RNA gene sequences. *Science* 226: 1965-1967.

HÖLLDOBLER, B. & E. O. WILSON. 1990. The fungus growers. Pp. 596-608 in Hölldobler, B. & Wilson, E. O. (eds.). *The Ants*. Harvard University Press, Cambridge.

HÖLLDOBLER, B. & E. O. WILSON. 1994. The Superorganism. Pp. 107-122 in Hölldobler, B. & Wilson, E. O. (eds.). *Journey to the Ants*. The Belknap Press of Harvard University Press, Cambridge.

HOWARD, J. J. 1987. Leafcutting ant diet selection: the role of nutrients, water, and secondary chemistry. *Ecology* 68: 503-515.

HOWARD, J. J., M. LAWRENCE HENNEMAN, G. CRONIN, J. A. FOX & G. HORMIGA. 1996. Conditioning of scouts and recruits during foraging by a leaf-cutting ant, *Atta colombica. Animal Behaviour* 52: 299-306.

HUBBELL, S. P., D. F. WIEMER & A. ADEJARE. 1983. An antifungal terpenoid defends a Neotropical tree *(Hymanaea)* against attack by fungus-growing ants *(Atta)*. *Oecologia* 60: 321-327.

HUBBELL, S. P., J. J. HOWARD & D. F. WIEMER. 1984. Chemical leaf repellency to an attine ant: Seasonal distribution among potential host plant species. *Ecology* 65: 1067-1076.

JAFFÉ, K. & P. E. HOWSE. 1979. The mass recruitment system of the leaf cutting ant *Atta cephalotes* (L.). *Animal Behaviour* 27: 930-939.

KACELNIK, A. 1993. Leaf-cutting ants tease optimal foraging theories. *Trends in Ecology and Evolution* 8: 346-348.

KACELNIK, A. & I. CUTHILL. 1990. Central place foraging in starlings (*Sturnus vulgaris*). II. food allocation to chicks. *Journal of Animal Ecology* 59: 655-674.

KARSLAKE, E. B. 2014. Do leaf-cutter ants (*Atta cephalotes*) exhibit crop leaf preferences when already transporting leaves? Animal Behavior Symposium, Princeton.

LEAL, I. R., R. Wirth & M. Tabarelli. 2014. The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified neotropical forests. *Biotropica* 46: 516–528.

MARTIN, M. M. 1970. The biochemical basis of the fungus-attine ant symbiosis. *Science* 169: 16-20.

MCGINLEY, M. A. 1984. Central place foraging for nonfood items: determination of the stick size-value relationship of housing building materials collected by eastern woodrats. *American Society of Naturalists* 123: 841-853.

MCNAMARA, J. M. 1982. Optimal patch use in a stochastic environment. *Theoretical Population Biology* 21: 269-288.

MUELLER, U. G. & N. GERARDO. 2002. Fungus-farming insects: multiple origins and diverse evolutionary histories. *Proceedings of the National Academy of Science, USA* 99:15247-15249.

MUELLER, U. G., J. POULIN & R. M. M. ADAMS. 2004. Symbiont choice in a fungus-growing ant (Attini, Formicidae) *Behavioral Ecology* 2: 357-367.

NICHOLS-ORIANS, C. M. 1991. Environmentally induced differences in plant traits: consequences for susceptibility to a leaf-cutter ant. *Ecological* 72: 1609-1623.

NICHOLS-ORIANS, C. M. & J. C. SCHULTZ. 1989. Leaf toughness affects leaf harvesting by the leaf-cutter ant, *Atta cephlotes* (L.) (Hymenoptera: Formicidae). *Biotropica* 21: 80-83.

OLSSON, O. & A. BOLIN. 2014. A model for habitat selection and species distribution derived from central place foraging theory. *Oecologia* 175: 537–548.

OLSSON, O., J. S. BROWN & K. L. HELF. 2008. A guide to central place effects in foraging. *Theoretical Population Biology* 74: 22–33.

ORIANS, G. R. 1980. Tests of central place foraging theory. Pp. 131-140 in Orians, G. R (ed.). *Some Adaptions of Marsh-nesting Blackbirds*. Princeton University Press, Princeton.

STARKOVICH, B. M. 2015. Optimal foraging, dietary change, and site use during the Paleolithic at Klissoura Cave 1 (southern Greece). *Journal of Archaeological Science* 52: 39-55.

PERKS, P. & L. MOORE. 2014. Leaf-cutter ants, *Atta cephalotes*, already transporting crop leaves tend to ignore forest plant leaves in smorgasbord trials. Abstracts of URS, 2 May 2014, University of Missouri-St. Louis, St. Louis.

ROCES, F. 1990. Olfactory conditioning during the recruitment process in a leaf cutting ant. *Oecologia* 83: 261-262.

ROCES, F. & J. A. NUNEZ. 1993. Information about food quality influences load-size selection in recruited leaf-cutting ants. *Animal Behaviour* 45: 135-143.

SAVERSCHEK, N., H. HERZ, M. WAGNER & F. ROCES. 2010. Avoiding plants unsuitable for the symbiotic fungus: learning and long term memory in leaf-cutting ants. *Animal Behaviour* 79: 689-698.

SCHOENER, T. W. 1979. Generality of the size-distance in models of optimal feeding. *The American Naturalist.* 114: 902-914.

SEAL, N. J. 2006. Self-organization and the superorganism: functional ecology of the obligate mutualism between a fungus gardening ant and its symbiont fungus. *Electronic Thesis and Dissertations.* Florida State University, Tallahassee. Paper 289.

SILVA, P. S. D., A. G. D. BIEBER, T. A. KNOCH, M. TABARELLI, I. R. LEAL, & R. WIRTH. 2013. Foraging in highly dynamic environments: leaf-cutting ants adjust foraging trail networks to pioneer plant availability. *Entomologcal Experimentalis et Applicata* 147: 110–119.

STEPHENS, D. W. & J. R. KREBS. 1986. Foraging Theory. Princeton University Press, Princeton.

WIRTH, R., H. HERZ, R. J. RYEL, W. BEYSCHLAG & B. HÖLLDOBLER. 2003. Herbivory of leaf-cutter ants: a case study on *Atta colombica* in the tropical rainforest of Panama. *Ecological Studies* 164.

Chapter 2

Ecological characterization of occupied and unoccupied habitats by the leaf-cutter ant (*Atta cephalotes*) at CEIBA Biological Center

Elizabeth B. Karslake

Leaf-cutters "benefited by the advent of European civilization. The ubiquitous Atta cephalotes...is specialized to live in forest gaps, and as a consequence it is able to invade subsistence farms and plantations from Mexico to Brazil."
In: The Ants, 1990, Hölldobler & Wilson, Pp. 597

Abstract

Leaf-cutter ant, *Atta cephalotes* nest placement is crucial to colony survival by facilitating access to profitable foraging locations. I tested the hypothesis that habitats occupied by active leaf-cutter ant nests differed structurally from unoccupied habitats. Measurements were made of habitat components in environments occupied by active ant nests and those without nests during summer 2014 at CEIBA Biological Center, Guyana. *Atta cephalotes* were more frequently encountered in human disturbed habitats, as significantly more nest entrances were clustered in active slash-and-burn agricultural plots. However, no active nests occurred in old second-growth forests. Sun exposure was significantly higher in sites with active nests than in unoccupied older second-growth forests. Additionally, woody and herbaceous plants in ant occupied and unoccupied habitats also differed in their diameters at breast height. Thus the hypothesis was corroborated because there were differences for quantitative ecological variables in ant occupied and unoccupied habitats. However, additional ecological variables should be measured in future studies to determine whether linear relationships exist for the number of ant nest entrances and habitat variables for disturbed, young, and mature second growth forests.

Introduction

Habitat occupancy and selection by dispersing organisms is dependent upon habitat quality. Thus, habitat selection models expect searching animals to assess a habitat's quality for availability of food, water, and shelter (Hildén 1965; Stamps 1987). The presence of conspecifics can indicate a good quality habitat, but too many conspecifics or heterospecifics can negatively influence individual fitness through competition (Connel 1961; Stamps 2001). Likewise, animals often avoid areas with high predation pressure that can further negatively impact fitness (Stamps 2001; Stephens & Peterson 1984). During the first phase of habitat selection the organism searches for a suitable habitat based on information imprinted from its natal home or information gained during the search process. Then, after assessing a habitat's quality an individual settles in the chosen habitat, and residency begins (Stamps 1987, 2001). An animal will stay in this habitat as long as its survival needs are meet and the animal has the physiological mechanisms to cope with any environmental difficulties (Connel 1961). If not then the search process begins again (Stamps 2001). Therefore, ecological variables of occupied habitats can be described, compared to variables of unoccupied habitats, and used to predict where populations of study organisms will occur (Connel 1961; Stamps 2001).

Many comparative studies indicate that ecological characteristics of disturbed sites can be beneficial to some types of organisms. For example, eastern whippoorwill (*Antrostomus vociferus*) abundance is higher in red and white pine forest sections clear-cut during the last 15 years (Tozer et al. 2014). These habitats have more shelterwood, and models predicted that whippoorwills could more easily spot insects by moonlight in larger cleared sections. Therefore, the presence of clear-cut forest sections can increase abundance of the threatened whippoorwills (Tozer et al. 2014). Likewise, the large blue butterfly's (*Phengaris teleius*) abundance is highest in farm plots in Hungry cleared yearly (Körösi et al. 2014). The host plant (*Sanguisorba officinalis*) of *P. teleius* was more abundant in fields cut once a year during September then plots cut twice a year, once a year in May, or not at all (abandoned plots). However *P. teleius* host ant (*Mymicra* sps.) abundance did not differ across field treatments. Körösi et al. (2014) concluded that proper management of farms once yearly during September was necessary to provide ample habitats for threatened *P. teleius.* Similarly, leaf-cutter ant occupancy is higher in young second-growth forests than old second growth forests (Farji-Brener 2001), likely due to the different ecological characteristics between habitats that they occupy and habitats they ignore. The palatable forage hypothesis predicts leaf-cutter ant colonies to be found in young second growth forests where pioneer plants are common (Farji-Brener 2001). Pioneer plants are mostly herbaceous species needing higher percent sun exposure, congregating in cleared forest sections, and are a large portion of leaf-cutter ant fungus diet. Another hypothesis suggests that founding queens purposely select cleared spaces with access to burrowing ground as well as pioneer plants (Farji-Brener 2001; Hölldobler & Wilson 1990).

At CEIBA Biological Center (CEIBA), Guyana forested areas are being converted to open habitats by slash-and burn agriculture, and consequently the number of active leaf-cutter ant nests also increases (Bourne & Bourne 2010). Furthermore, leaf-cutter ant occupancy has profound affects on surrounding plant communities including decreased survival of woody plants and increased sunlight exposure as canopy foliage is removed by foraging ants (Wirth et al. 2003). Therefore my aim for this thesis chapter was to measure ecological variables of an upland seasonal second growth forest in Guyana and adjacent slash-and-burn or swidden farms to identify variables association with the presence or absence of leaf-cutter ant nests. I tested the hypothesis that habitats with active *Atta cephalotes* nests differ structurally from unoccupied habitats, by comparing ecological variables of these two habitat types. I predicted that: prediction one (P_1), there will be more ant nest entrances in disturbed habitats such as slash-and-burn farms than in old-second growth forest habitats; P_2, habitats with active ant nests will have higher sun exposure (a proxy for canopy cover) compared to habitats without nests; and P_3, plants in habitats unoccupied by ants will have a greater mean diameter at breast-height (DBH) than plants in habitats occupied by ants.

Methods

Study species: Leaf-cutter ants belong to the genus *Atta*, and all estimated 15 to 17 species are found throughout the New World ranging from Texas to Argentina (Hölldobler & Wilson 1994). The study species, *Atta cephalotes* (Fig. 1)*,* ranges from Mexico to the Northern regions of Brazil (Hölldobler & Wilson 1990). Castes are assigned by body size including: (1) majors as soldiers patrol among fungus gardens and foraging ants to ensure safety of the colony; (2) media as foragers; and (3) minima, of several sizes, work inside the gardens carrying for the fungus and brood (Hölldobler & Wilson 1994). However, minimas occasionally ride on leaves carried by media, and occasionally majors, to ward off phyroid flies (Wirth et al. 2003). Foragers harvest substrates from suitable plants and carry loads back over trails well maintained with pheromones (Jaffé & Howse 1979; Wirth et al. 2003). Leaves are deposited by the media in a pile for the minima to process, mulch, and insert into fungus gardens (Hölldobler & Wilson 1990).

Mature colonies contain between 10,000 to several million individuals (Hölldobler & Wilson 1990), and *Atta cephalotes* nests at CEIBA are visible because of displaced soil, leaves, and white sand mounded up around entrances. Each colony has many entrances that are used for different purposes. A few entrances only access dumping sites where workers place nest debris, including rejected fungal substrates, and some openings provide ventilation by improving air flow through the nest (Hölldobler & Wilson 1990). Other entrances are used to bring in leaf-clippings, but ants are most likely to use entrances with access to trails leading to desirable foraging patches. Colonies are extensive, penetrating at least 3 m into the ground and may be as deep as 9 m. Here, nest chambers are used as fungus gardens, and as dumping sites of spent mulch (Hölldobler & Wilson 1990). Colonies at CEIBA were at least 5 m in diameter and aggregated in disturbed areas that included roads, forest paths, and farms (Bourne & Bourne 2010; Karslake 2014). Foraging trails lead to vegetation patches, and at CEIBA, many trails lead to the center of gardens or farms containing citrus trees (*Citrus* sp.), avocado pear (*Persea americana*), and other cultivars (Karslake 2014).

Atta cephalotes mark ever changing trail networks with pheromones and the presence of a trail is mostly effected by the availability of pioneer plants (Silva et al. 2013). Pheromones are secreted from ants' poison sacs and have several functions (Hölldobler & Wilson 1990; Wirth et al. 2003). One function is to act as an orientation cue to help recruited foragers locate chosen vegetation patches, and these markings are placed on both the ground and plants (Wirth et al. 2003). Concentrations of pheromones laid down by scouts indicate quality of a foraging patch. Higher pheromone concentrations are more likely to elicit recruitment behavior than lower concentrations (Jaffé & Howse 1979). Recruits often apply fresh pheromones to old trails, and scouts lay new trails leading to new vegetation patches (Wirth et al. 2003). Ants can recognize odors of plant types, and recruits may possibly be conditioned by odors of substrates scouts bring back to the nest (Roces 1990). However, ants rely upon previous experience to determine wheatear a leaf type is suitable for their fungal cultivar (Saverschek et al. 2012).

Leaf-cutter ants usually prefer leaves lacking unpalatable secondary compounds such as terpenoids, tannins, and other non-polar extractables (Hubbell et al. 1983; Hubbell et al. 1984; Howard 1987). Like other leaf-cutter ants, *A. cephalotes* forage for leaves, flowers, and fruits, but during dry seasons will also collect lichens, mosses, wood, and dead insects (Leal & Oliveria 2000). Leaf-cutters selectively forage from favored plants, but as the seasons progress foragers gather fewer leaves from the same plants. Possibly as the rainy season changes to dry, the leaf chemistry changes and leaves develop more defensive compounds (Howard et al. 1996; Hubbell et al. 1984). All *Atta* spp. are considered agricultural pests because they incorporate considerable amounts of crop leaves in the mulch fed to their mutualistic fungi (Cherrett 1968; Hölldobler & Wilson 1990). This has lead Wirth et al. (2003) to speculate that leaf-cutters prefer cultivated plants as these usually have significantly lower levels of defensive mechanisms then found in native plants (Chen et al. 2015; Shang et al. 2014). This discussion of the characteristics of *A. cephalotes* suggests that it is tractable for ecological studies associated with habitat occupancy and foraging behavior.

Figure 1. Major, media, and minima caste phenotypes of *Atta cephalotes* cutting up a cashew (*Anacardium occidentale*) leaf for transport to the nest.

Figure 2. No active *A. cephalotes* nests were ever found in old second growth or primary forests (seen in back of swidden plots). However, high densities of leaf-cutter ant nests dotted slash-and-burn agriculture plots (seen up front).

Study site: This study was conducted at CEIBA Biological Center, Madewini, Guyana (06° 29.928′ N, 58° 13.111′ W). CEIBA was founded in 1993 by Dr. Godfrey Bourne as a research center of biodiversity for Guyanese and international students and scientists. The study site is situated between a highly eroded peneplain with an upland seasonal old second-growth forest (Fig. 2) and a flooded mora forest in the floodplain (Bourne & Bourne 2010). There is a variety of wildlife due to the proximity of the Madewini River. However, in recent years lands have become more developed as houses and farms were built, thus leading to increased encounter rates between wildlife and humans (Bourne & Bourne 2010). A common farming practice adjacent to CEIBA is slash-and-burn cultivation, where subsistence farmers clear-cut sections of forest and torch vegetation after it dries, crops are planted for a few years, and the swiddens abandoned and the process is repeated elsewhere (Fig. 2; Bourne & Bourne 2010). Increases in swidden fields, white sand mining, and development of Splashmins Ecocampground, Water Park and Luxury Villas at Madewini, has greatly increased open habitats conducive to population explosions of many ant species, but especially *A. cephalotes* (Bourne & Bourne 2010). Locals complain that leaf-cutter ants, known vernacularly as druggas or cushie ants, strip leaves from their cultivated plants so often that many cultivars do not have a chance to grow. For example, during this study it was common for leaf-cutter ants to completely defoliate lemon trees overnight (E.B. Karslake. pers. obs.).

Before recording habitat variables, locations of ant colonies were noted, and I determined whether neighboring colonies were indeed separate nests through aggression assays (Karslake 2014; Vilela & Howse 1986). Two ants about the same size (one from each colony) were caged in 1L clear plastic aquaria so they could interact. If interactions were aggressive then ants were considered to be from different colonies, but if the interaction was non-aggressive then the two individuals were nest mates. Aggressive behavior is indicated by an ant spreading its mandibles and legs, biting the other individual while quickly waving its antennae (Vilela & Howse 1986).

Habitat sampling protocols: Here I focused on ecological measurements (Wirth et al. 2003) of sun exposure, plant diameter at breast height (DBH), and number of active ant nest entrances to elucidate association with presence or absence of leaf-cutter ant nests. From 8-11 August 2014 measurements were collected along transects in two habitat types (Wiens 1969). The first habitat type was old second-growth forests, represented by a 50 year old upland seasonal forest. While the disturbed forests were represented by a swidden farm cut from the same 50 year old recovering forest. In total, there were eight transects (four of each in the two sampled habitats) set 50 m from each other running North to South for 400 m and designated A, B, C, D.

Sampling locations along transects were determined by randomly drawing three numbers from a brown paper bag (Wiens 1969). The first drawn number indicated the distance along the transect that researchers sampled. If the last digit of the second random number ended in an odd digit, then sampling was done on the left side, and if it was an even digit sampling was done on the right side. The third number determined the distance travelled perpendicular to the transect. Sampling was done at these locations, and there were 50 sampling sites for each location. Light intensity was measured in lux and compared to unobstructed open sky light intensity measured simultaneously and each converted to a percentage of open sky illumination as sun exposure, a proxy for canopy cover. Then, DBH (mm) of the closest plant to the sampling point was recorded, and leaf-cutter nest entrances tallied.

Statistical Analysis: To test P_1 that more ant nest entrances are in swidden habitats, I used the nonparametric Mann-Whitney *U*-test (two-tailed, $\alpha = 0.05$) because the data violated the assumptions of Gausian distribution and equality of variances for the parametric t-test (Whitlock & Schluter 2009). Likewise, Mann-Whitney *U*-tests tests (two-tailed, $\alpha = 0.05$) were used to determine whether, P_2 and P_3 differed by treatments, ant occupied and ant unoccupied habitats again because the assumptions for parametric t-tests were violated (Whitlock & Schluter 2009). Statistical tests and graphing were done by using SigmaPlot 11 statistical package (Systat Software 2008).

Results

Atta cephalotes nest entrances were more common in swiddens, human disturbed plots than undisturbed old second growth forests ($U = 0$, $p < 0.001$, $n = 50$; Fig. 3). The median number of nest entrances on disturbed plots was 2, but old-second growth forests had a median of zero nest entrances. Swiddens occupied by *A. cephalotes* nests were characterized by higher sun exposures compared to old secondary forest habitats unoccupied by ant nests (Fig. 4; $U = 145$, $p < 0.001$, $n = 50$). Diameters of plants in unoccupied second growth forests were significantly thicker than plants from occupied leaf-cutter ant habitats (Fig. 5; $U = 39$, $p < 0.001$, $n = 50$).

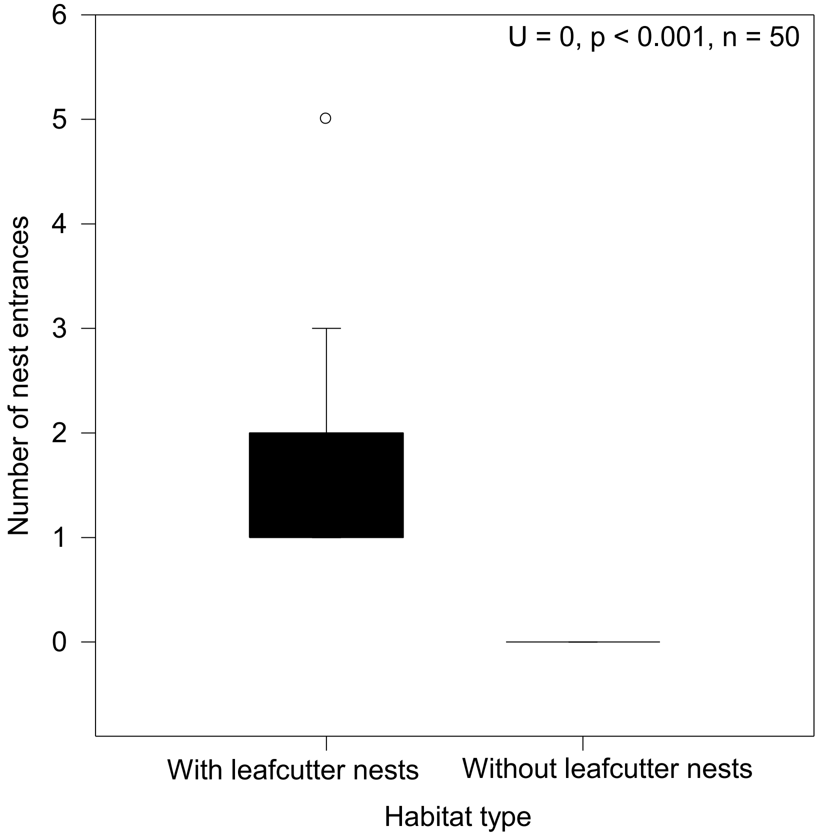


Figure 3. Disturbed habitats containing slash-and-burn farms had significantly more nest entrances than old-second growth forest habitats where no active leaf-cutter ant nests were found.

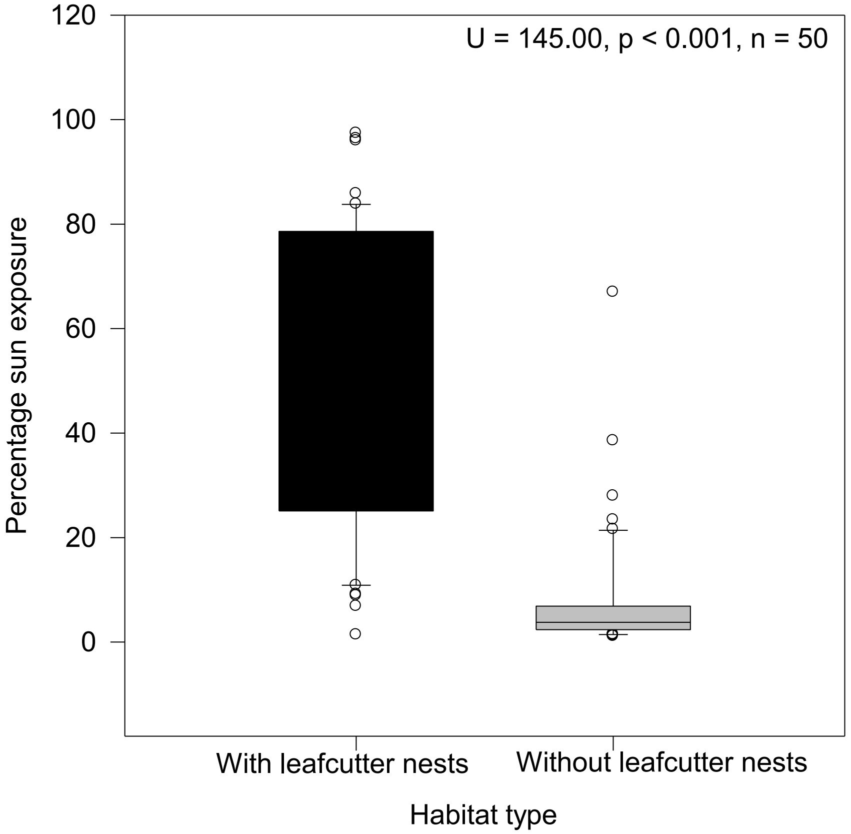


Figure 4. Percent sun exposure of sampled sites in habitats with either with active or inactive *A. cephalotes* nests, showing significantly more percent sun exposure on sties with ants.

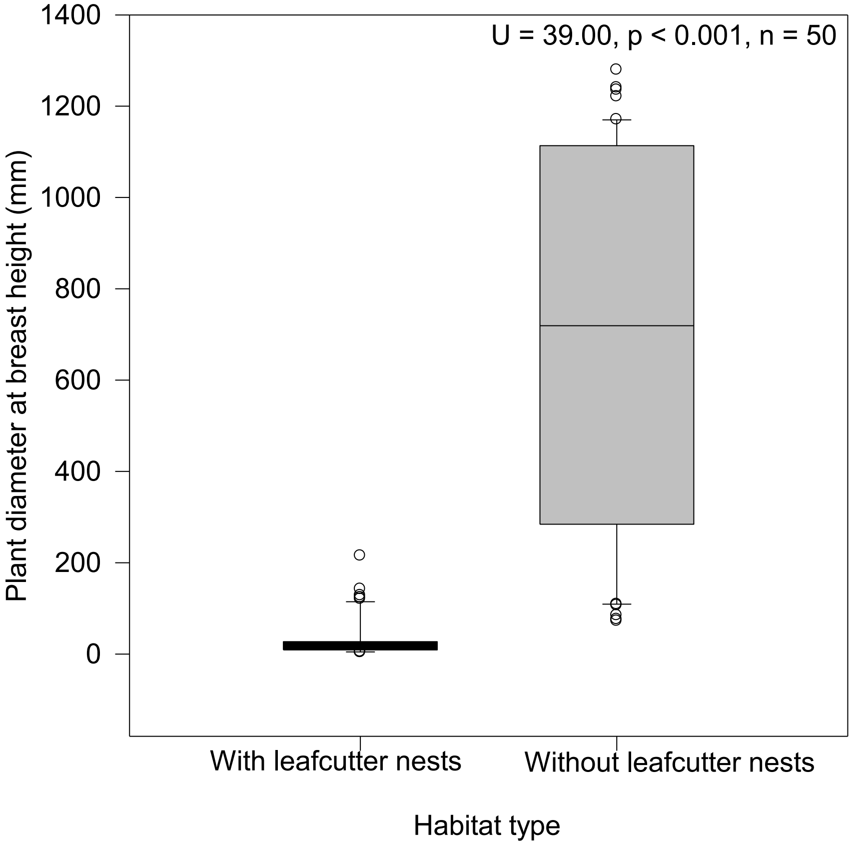


Figure 5. Plant diameter at breast height (mm) was significantly larger in habitats without leaf-cutter ant nests than habitats with ants.

Discussion

Habitats occupied by leaf-cutter ant nests differed structurally from unoccupied habitats. *Atta cephalotes* nests were clustered in recently disturbed habitats as ant nest entrances were found only in swidden plots, but there were no nests in old second growth forests. This was strong evidence in support of P_1, that ant nest are more common in human modified habitats. Additionally, habitats with leaf-cutter ants were associated with smaller canopy cover reflected by higher percent sun exposure, and were characterized by smaller plant DBHs. This was evidence in support P_2, that leaf-cutter ant occupancy of habitats is associated with little canopy cover. Finally, P_3, that plants in habitats occupied by leaf-cutter ants have lower DBHs was supported. These disturbed habitats were dominated by pioneer plants that are found in cleared forest patches; these comprise the majority of the plant species leaves harvested by leaf-cutter ants (Farji-Brener 2001; Leal et al. 2014; Silva et al. 2013; Vassconcelos & Cherrett 1995).

Atta cephalotes habitat selection and occupancy was consistent with the palatable forage hypothesis that these habitats are associated with pioneer plant abundance (Farji-Brener 2001; Leal et al. 2014). A meta-analysis conducted by Farji-Brener (2001) suggested that *Atta* spp. prefer pioneer plants over leaves from shade-tolerant forest species. Indeed, during a leaf pick-up (leaf preference) assay *A. cephalotes* choose leaves of pioneer plants significantly more often than shade tolerant species (Farji-Brener 2001). Thus, pioneer plants are critical to the maintenance of healthy populations of leaf-cutter ants (Leal et al. 2014); for example, in Brazil, the proportions of leaf-cutter ants and pioneer plants increase from mature to old to young second growth forest habitats (Vassconcelos & Cherrett 1995). Densities of *Atta* nests can increase up to

30 times in young secondary forests compared to mature forests, while *Acromyrmex* nests increase by 20 percent. Therefore, deforestation in Amazonia increases the geographical range of leaf-cutter ant species, and high leaf-cutter ant prevalence is evidence of habitat disturbance, such as the swidden plots adjacent to CEIBA Biological Center (Vassconcelos & Cherrett 1995; Wirth et al. 2003).

Leaf-cutter ant residency and their herbivory profoundly alters plant communities over the long-term in many ways, for example, a common effect of leaf-cutter ant foraging is removal of preferred plant canopy foliage thereby increasing light transmission to the forest floor (Saha et al. 2012; Terbourgh et al. 2006; Wirth et al. 2003). The increased illumination facilitates conditions conducive to the promotion of luxuriant pioneer plant growth in understory gaps (Leal et al. 2014). However, leaf-cutter ant presence can decrease plant diversity and sapling survival (Terbourgh et al. 2006; Wirth et al. 2003). Terbourgh et al. (2006) found that smaller islands at Lage Guri, Venezuela had larger leaf-cutter ant populations but only 25% of the flora species found on larger islands, and with a negative correlation between leaf-cutter ant presence and decreased sapling recruitment on smaller islands. In another study, short statured trees dominated some habitats in an Amazon rainforest, and those within 10 m of *A. cephalotes* nests had access to recycled plant nutrients (Saha et al. 2012). However, saplings, did not have access to these nutrients and there was lower seedling abundance and plant species richness within 10 m of active ant nests (Saha et al. 2012).

In conclusion, I found support for the hypothesis that habitats occupied by leaf-cutter ants differed structurally from unoccupied habitats. My results suggested that *A. cepatlotes* selected human altered habitats with smaller canopy cover and lower plant

DBHs. However, future studies should expand sampling of swidden, second growth, and mature forests at CEIBA Biological Center and adjoining landscapes to correlate *A. cephalotes* habitat occupancy with quantitative ecological variables, and these should include area of ant nest size, number of nest entrance mounds, colony numbers, pioneer plant density, percent sunlight exposure, and woody plant DBH. While previous research examined the effects of leaf-cutter ant occupancy in forested habitats, I agree with Leal et al. (2014) that additional studies are needed to elucidate long term impacts of leaf-cutter ants in human altered forests.

Literature Cited

BOURNE, G. R. & C. BOURNE. The biological station and its programs. Pp. 43-45 in Bourne, G. R. & Bourne, C. (eds.). *The CEIBA Reader*. Yerfdog Publishing, St. Louis.

CHEN, Y. H., R. GOLS & B. BENREY. 2015. Crop domestication and its impact on naturally selected trophic interactions. *Annual Review of Entomology* 7: 35-58.

CHERRETT, J. M. 1968. The foraging behaviour of *Atta cephalotes* L. (Hymenoptera, Formicidae). *Journal of Animal Ecology* 37: 387-403.

CHERRETT, J. M. 1986. Chemical control and bait formulations for leaf-cutting ants. Pp. 357-368 in Lofgren, C. S. & Vander Meer, R. K. (eds.). *Fire Ants and Leaf-Cutting Ants Biology and Management*. Westview Press, Boulder.

CONNEL, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus Stellatus*. *Ecology* 42: 710-723.

FARJI-BRENER, A. G. 2001. Why are leaf-cutting ants more common in early secondary forests than in old-growth tropical forests? An evaluation of the palatable forage hypothesis. *OIKOS* 92: 169–177.

HILDÉN, O. 1965. Habitat selection in birds: A review. *Annales Zoologici Fennici* 2: 53-75.

HÖLLDOBLER, B. & E. O. WILSON. 1990. The fungus growers. Pp. 596-608 in Hölldobler, B. & Wilson, E. O. (eds.). *The Ants*. Harvard University Press, Cambridge.

HÖLLDOBLER, B. & E. O. WILSON. 1994. The Superorganism. Pp. 107-122 in Hölldobler, B. & Wilson, E. O. (eds.). *Journey to the Ants*. The Belknap Press of Harvard University Press, Cambridge.

HOWARD, J. J. 1987. Leafcutting ant diet selection: the role of nutrients, water, and secondary chemistry. *Ecology* 68: 503-515.

HOWARD, J. J., M. LAWRENCE HENNEMAN, G. CRONIN, J. A. FOX & G. HORMIGA. 1996. Conditioning of scouts and recruits during foraging by a leaf-cutting ant, *Atta colombica. Animal Behaviour* 52: 299-306.

HUBBELL, S. P., D. F. WIEMER & A. ADEJARE. 1983. An antifungal terpenoid defends a Neotropical tree *(Hymanaea)* against attack by fungus-growing ants *(Atta)*. *Oecologia* 60: 321-327.

HUBBELL, S. P., J. J. HOWARD & D. F. WIEMER. 1984. Chemical leaf repellency to an Attine ant: seasonal distribution among potential host plant species. *Ecology* 65: 1067-1076.

JAFFÉ, K. & P. E. HOWSE. 1979. The mass recruitment system of the leaf cutting ant *Atta cephalotes* (L.). *Animal Behaviour* 27: 930-939.

KARSLAKE, E. B. 2014. Do leaf-cutter ants (*Atta cephalotes*) exhibit crop leaf preferences when already transporting leaves? Animal Behavior Symposium, Princeton.

KÖRÖSI, A., I. SZENTIRMAI, P. BATARY, S. KÖVER, N. ÖRVÖSSY & L. PEREGOVITS. 2014. Effects of timing and frequency of mowing on the threatened scarce large blue butterfly-A-fine-scale experiment. *Agriculture, Ecosystems, and Environment* 196: 24-33.

LEAL, I. R. & P. S. OLIVERIA. 2000. Foraging ecology of attine ants in a Neotropical savanna: seasonal use of fungal substrate in the cerrado vegetation of Brazil. *Insectes Sociaux* 47: 376-382.

LEAL, I. R., R. Wirth & M. Tabarelli. 2014. The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified neotropical forests. *Biotropica* 46: 516–528.

ROCES, F. 1990. Olfactory conditioning during the recruitment process in a leaf-cutting ant. *Oecologia* 83: 261-262.

SAHA, A. K., K. S. CARVALHO, L. S. L. STERNBERG & P. MOUTINHO. 2012. Effect of leaf-cutting ant nests on plant growth in an oligotrophic Amazon rain forest. *Journal of Tropical Ecology* 28: 263–270.

SAVERSCHEK, N., H. HERZ, M. WAGNER & F. ROCES. 2010. Avoiding plants unsuitable for the symbiotic fungus: learning and long term memory in leaf-cutting ants. *Animal Behaviour* 79: 689-698.

SHANG, Y., Y. MA, Y. ZHOU, H. ZHANG, L. DUAN, H. CHEN, J. ZENG, Q. ZHOU, S. WANG, W. GU, M. LIU, J. REN, X. GU, S. ZHANG, Y. WANG, K. YASUKAWA, H. J. BOUWMEESTER, X. QJ, Z. ZHANG, W. J. LUCAS, S. HUANG. 2014. Biosynthesis, regulation, and domestication of bitterness in cucumber. *Science* 346: 1084-1088.

SILVA, P. S. D., A. G. D. BIEBER, T. A. KNOCH, M. TABEARELLI, I. R. LEAL & R. WIRTH. 2013. Foraging in highly dynamic environments: leaf-cutting ants adjust foraging trail networks to pioneer plant availability. *Entomologcal Experimentalis et Applicata* 147: 110–119.

STAMPS, J. A. 1987. Conspecific attraction and aggregation in territorial species. *American Naturalist* 131: 41-58

STAMPS, J. A. 2001. Habitat selection by dispersers: integrating proximate and ultimate approaches. Pp. 230-242 in Clovert, J., Danchin, E., Dhondt, A. A. & Nichols, J.D. *Dispersal.* Oxford University Press, Oxford.

STEPHENS, P. W. & R. O. PETERSON. 1984. Wolf-avoidance strategies of moose. *Ecography* 7: 239-244.

SYSTAT SOFTWARE. 2008. SigmaPlot 11.0 User's Guide. Systat Software, Inc., San Jose.

TERBOURGH, J., K. FEELEY, M. SILMAN, P. NUNEZ & B. BALUKJIAN. 2006. Vegetation dynamics of predator-free land-bridge islands. *Journal of Ecology* 94: 253–263.

TOZER, D. C., J. C. HOARE, J. E. INGLIS, J. YARASKAVITCH, H. KITCHING & S. DOBBYN. 2014. Clearcut with seed trees in red pine forests associated with increased occupancy by Eastern Whip-poor-wills. *Forest Ecology and Management* 330: 1–7.

VASCONCELOS, H. L. & J. M. CHERRETT. 1995. Changes in leaf-cutting ant populations (Formicidae: Attini) after the clearing of mature forest in Brazilian Amazonia. *Studies on Neotropical Fauna and Environment* 30: 107-113.

VILELA, E. F. & P. E. HOWSE. 1986. Territoriality in Leaf-Cutting Ants, *Atta* spp. Pp. 159-171 in Lofgren, C. S. & Vander Meer, R. K. (eds*). Fire Ants and Leaf-Cutting Ants.* Westview Press, Boulder.

WHITLOCK, W. C. & D. SCHLUTER. 2009. Comparing two groups: the Mann - Whitney *U* test. Pp. 337-342 in Whitlock, W. C. & Shluter, D. (eds*). The Analysis of Biological Data*. Roberts and Company Publishers, Greenwood Village.

WIENS, J. A. 1969. Proceedures and techniques. Pp. 20-29. *An Approach to the Study of Ecological Relationships Among Grassland Birds*. Ornithological Monographs No. 8. The American Ornithologists Union.

WIRTH, R., H. HERZ, R. J. RYEL, W. BEYSCHLAG & B. HÖLLDOBLER. 2003. Herbivory of leaf-cutter ants: a case study on *Atta colombica* in the tropical rainforest of Panama. *Ecological Studies* 164.

Chapter 3

Leaf-cutter ants, *Atta cephalotes*, already transporting leaves to their nests at CEIBA do not abandon their fragments for domesticated cultivar leaves.

Elizabeth B. Karslake

We arose one morning and found our garden defoliated...Into a hole...ants...were carrying bits of our cabbage, tops of the carrots, the beans-in fact our entire garden was going down the hole. I could see the grinning face of the toothless Miskito Indian. The Wiwis [leaf-cutters] had come.
-V. Wolfgang von Hagen **In: The Ants, 1990, Hölldobler & Wilson, Pp. 596**

Abstract

Leaf-cutter ants are noted agricultural pests throughout the New World. They harvest leaves and other plant organs, and feed these to their cultivated symbiotic fungi. Ants prefer leaves with lower levels of plant specialized metabolites that discourage herbivory, but many of these are removed during domestication of cultivated plants. I therefore posited that leaf-cutter ants (*Atta cephalotes*) in Guyana prefer crop leaves over forest leaves because forest plants have higher levels of non-polar compounds (volatiles). Leaves offered from farm cultivars were preferred by ants over forest leaves. Furthermore, acceptance and rejection counts of leaf types indicated avoidance of a forest type, the bush cherry (*Eugenia lambertiana*), but acceptance of orange (*Citrus sinensis*) and West Indian almond (*Terminalia catappa*) leaves thereby demonstrating preference for crop leaves. Colony preference variation was detected, and while native plant species collectively contained more plant specialized metabolites, volatiles were detected in orange leaves but were not in bush cherry leaves. However, I did not measure leaf polar compounds. Thus, other factors such as learned colony preferences for particular plant types may determine a leaf-cutter ant colony's plant preferences.

Introduction

Leaf-cutter ant foraging preferences are partially affected by a colony's relationship with its symbiotic fungus gardens. Leaf-cutter ants select leaves with nutrients (such as sugars), moisture, and possibly salts that are needed by the fungus and ants (Coblentz et al. 2013; Howard 1996). Other factors of leaf quality include the presence of competitive endophytic fungi and leaf tenderness, which makes it easier for ants to cut leaves (Chavarria-Pizarro et al. 2012; Nichols-Orians & Schultz 1989). By weeding out competitive fungus, providing the necessary amino acids, and feeding the fungus gardens high quality vegetation fragments, leaf-cutter ant colonies are rewarded as fungus gardens quickly increase in size (Howard et al. 1996; Martin 1970; Seal 2006; Wirth et al. 2003). However, when fed lower quality items, the fungus growth rates are slower; exhibiting negative feedback (Seal 2006). Worker ants then learn to reject items associated with the growth decline (this is called delayed rejection) as these are now considered unpalatable (Saverschek et al. 2010). Currently, it is thought that information about unpalatable leaf types is conveyed amongst workers inside the gardens (Wirth et al. 2003). During this process, foragers then learn to recognize various palatable plant species and avoid unacceptable types (Saverschek et al. 2010).

Ants prefer leaves with no, or very low amounts of plant secondary metabolites which are harmful to fungus cultivar (Howard 1987; Hubbell et al. 1984; Nichols-Orians 1991). Of 42 randomly sampled species from a dry forest in Costa Rica many contained extractable repellents (Hubbell et al. 1984). Occurrences of extractable repellents (especially non-polar volatiles) correlate positively with plant avoidance by wild *A. cephalotes* (Hubbell et al. 1984), and isolation of extractables showed many to be terpenoids which can be highly toxic to fungus (Hubbell et al. 1984). *Atta cephalotes* in a previous study by Hubbell et al. (1983) likewise avoided leaves of *Hymenaea courbail* because of the presence of terpenoid compounds in the leaves. When this terpenoid, caryophyllene epoxide, was sequestered and added to palatable leaves ants rejected these previously acceptable samples, displaying delayed rejection (Hubbell et al. 1983). Possibly leaf-cutter ants prefer crop leaves over forest leaves as many crops, like domestic cucumber (*Cucumis sativus*), lack defensive mechanisms (such as non-polar volatiles) found in native species (Chen et al. 2015; Shang et al. 2014; Wirth et al. 2003). Indeed several species demonstrate a preference for crop leaves including *A. texana and A. cephalotes* (Waller 1986; Hölldobler & Wilson 1990). *Atta cephalotes* at Karanmbu Trust, Guyana strongly prefer crop leaves over native forest leaves (Burton & Holden 2012; Perks & Moore 2014). Foraging ants offered whole crop leaves readily abandoned forest leaves they were carrying for these leaves (Burton & Holden 2012). Also, there was a shorter handling time of preferred leaves with increasing handling time of less preferred leaves.

Therefore, I tested the hypothesis that leaf-cutter ants already transporting leaves preferred leaves of crops over native forest plants because many crops lack non-polar compounds found in native species that repel leaf-cutter ants (Howard 1987; Wirth et al. 2003). Specifically, I focused on *A. cephalotes* at CEIBA Biological Center, Guyana because they are common at human disturbed sites and are serious agricultural pests (Bourne & Bourne 2010; Hölldobler & Wilson 1990). To test the above hypothesis I offered cultivated and forest leaves arranged in a randomized smorgasbord to foraging ants. I measured latency time for ants to discover leaf samples, and leaf handling time as ants investigated, cut up, and carried them to their nest. Then, I measured percent acceptance or rejection of leaf samples over repeated smorgasbord offerings. In addition, leaf samples were taken to the University of Missouri-St. Louis (UMSL) where I determined the presence and types of non-polar leaf metabolites using gas chromatography/mass spectrometry (GC-MS). I predicted: P_1 preferred leaves in the randomized smorgasbord will be indicated by shorter discovery and handling times of leaf samples; P_2 preferred leaves have higher percent acceptance; P_3 crop leaves are preferred over forest leaves; P_4 preferred samples have fewer total non-polar extractables.

Methods

Smorgasbord presentation of forest and crop leaves: Observations were made during nights and overcast days when ants were actively foraging, and data were collected from 29 June to 5 August 2013 and from 3 June 2014 to 10 July 2014. To determine plant acceptability, I simultaneously presented a smorgasbord of eight leaf samples 2.5 cm in diameter (four cultivated and four forest species each) to the ants in a randomized block design (Burton & Holden 2012). This randomized smorgasbord was presented only to ants actively transporting leaves back to the nest along the foraging trail, and leaf samples were arrayed directly in line of ants (Burton & Holden 2012). The following species were included in the smorgasbord design, orange (*Citrus sinensis)*, cashew *(Anacardium occidentale)*, avocado (*Persea americana)*, west indian almond (*Terminalia catappa)*, bush cherry (*Eugenia lambertiana*), dukka (*tapiria marchandii*), *Anthurium* sp, and ginger *(Zingiber* sp*)*. However, to control for leaf quality I picked mature leaves every two days (Howard et al. 1996). Dry and brittle leaves were avoided, and older leaves with epiphylic mosses and lichens were likewise avoided. Many young leaves were avoided as these included different classes of volatiles than mature leaves (Azam et al. 2013).

Measured variables included, discovery time in seconds (s) i.e., latency time for foraging ants to discover the leaf samples, handling time (s) of samples as ants investigated, cut up, and carried away leaf disks to their nests. Preferred leaves were carried into the nest, but rejected samples were dragged off the roadways by foraging ants, or completely ignored (Karslake 2014). Handling time ended after a leaf disk was completely accepted or rejected (Burton & Holden 2012). However, smorgasbord trials were terminated after 3 h and remaining leaves marked as rejected with a handling time of 3 h. This was done because after 3 hours ants often switched to another trail leading to a better vegetative patch, resulting in the older foraging trails (used for the randomized smorgasbord trials) being abandoned. In other instances, after 2-3 h ants would finish foraging for the night and closed up the nest entrances. The following morning, 12 h later, I checked each nest and noted if any samples rejected during the night had later been accepted. Mostly, samples rejected during the night remained rejected 12 h later, but occasionally one lone worker would be seen harvesting a sample from a previous smorgasbord trial. Therefore, I measured the percentage of time a leaf sample (treatment) was accepted or rejected 3 h after a smorgasbord trial and again 12 h after the trial ended. Each ant nest was sampled once per day or night for five repeated trials.

Measuring leaf defensive compounds: Leaf samples were collected in the field, preserved in liquid nitrogen, and later analyzed to determine leaf volatiles concentrations using gas chromatography and mass spectrometry (GC-MS). Analyses were done in Dr. Rudolph Winter's lab at the University of Missouri-St. Louis to identify polar leaf metabolites present in leaf samples. I followed a protocol developed by Winter's lab by first cutting up leaves into 3 cm by 7 cm fragments. Volatiles from samples then were absorbed into a 2 mL 50:50 solution of ethanol and chloroform in a closed glass container to prevent evaporation. After two or three days of soaking I tested samples for presence volatiles.

For GC-MS analysis 2µl of ethanol/chloroform with extracted leaf volatiles were inserted into the GC instrument column where it was processed and later transferred to the mass spectrometry for analysis (Kamthan et al. 2012). Samples were injected at 30°C, but inside the machinery the samples ramped up to 250°C. Inside temperature and sample analysis was regulated by the GC-MS CSS and computer program. Enhanced Data computer programs also helped to identify each leaf volatile based on peak areas, normalization, and internal standards.

Data analysis: To assess differences among leaf sample discovery times for repeated trials I used a Friedman Repeated Measures Analysis of Variance on Ranks in SigmaPlot 11 because data distribution did not pass the Shapiro-Wilk Normality Test. I investigated changes in median discovery times over 5 repeated smorgasbord trials for 28 colonies (Scheiner & Gurevitch 2001). Likewise, a Friedman Repeated Measures Analysis of Variance on Ranks was used to compare medians of handling times for leaf samples of the 28 colonies over 5 repeated smorgasbord trials (Scheiner & Gurevitch 2001). Since differences among treatment groups were significant, I used a Tucky post-hoc test to asses which comparisons amongst colonies were significant.

To calculate significance of leaf sample acceptance and rejection counts 3 h after initiation of smorgasbord I used a χ^2 goodness-of-fit in SigmaPlot 11 ($\alpha = 0.05$). This χ^2 indicated if there were differences among observed and expected counts for each eight leaf treatments (Whitlock & Shluter 2009). In total there were 194 accept/reject counts for all 8 leaf treatments. A χ^2 goodness-of-fit was used for leaf sample accept/reject counts 12 h after smorgasbord initiation ($\alpha = 0.05$; Whitlock & Shluter 2009).

Results

Leaf preference: Preference for a particular leaf type was not discerned by discovery time (Fig. 1) as there were no significant differences ($p = 0.418$). However, there were significant differences among leaf sample handling times ($p < 0.001$). However, of all 40 comparisons only two pairs contributed to the overall significance. These pairs were colonies 29 and 1; and colonies 24 and 1. Therefore, leaf sample preference could be define by handling time but not discovery time. Sample disk acceptance or rejection for each plant type 3 h after the start of the smorgasbord was significantly different (Fig. 3a; $\chi^2 = 141.959$, df = 7, $p < 0.001$). The greatest proportion of total difference came from almond (20% greater than the expected value), orange (18% greater than the expected value), and bush cherry (17% less than the expected value). Likewise, differences among sample percent acceptance or rejection 12 h after the start of the smorgasbord were significant (Fig. 3b; $\chi^2 = 147.537$, df = 7, $p < 0.001$). Here, the greatest proportion of total difference came from almond (19%) and orange (19%) as these sample were accepted more often than rejected, but bush cherry (18%) was rejected more than expected.

Leaf volatiles: I observed 3 volatiles in leaves from cultivated species and 6 volatiles in leaves from native plants used in the randomized smorgasbord tests (Table 1).

Collectively native species contained more volatiles than cultivated species. 1R-.alpha.-pinene was recovered from cashew and ducka, and ß-pinene from orange, ginger, and ducka. Pinene is named because of its presence in pine resin and is widely used as an insect repellent (El Amine Dib et al. 2015). Limonene, also recovered from the orange leaf, is common in mature citrus species, giving citrus fruits its' characteristic fragrance, and is used as a biological insecticide (Azam et al. 2013). However, west indian almond, avocado pear, and bush cherry did not contain any observable volatiles possibly because these leaves were too waxy to resolve any definite compounds using this GC-MS protocol. Two fatty acids, n-Decanoic acid and 8, 11, 14-Eicosatrienoic acid, (Z,Z,Z), were found in ducka. contained 1-Heptatriacotanol. Ginger contained a second volatile (11-hexadecenal).

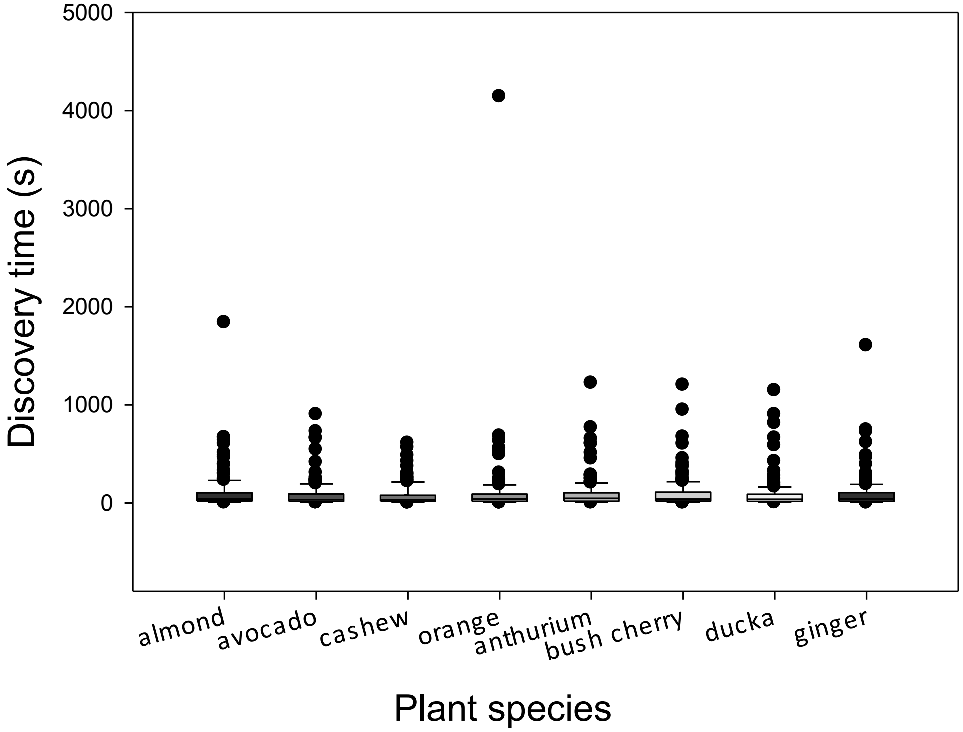


Figure 1. Median discovery times for leaf type treatments presented during the smorgasbord trials were non-significant.

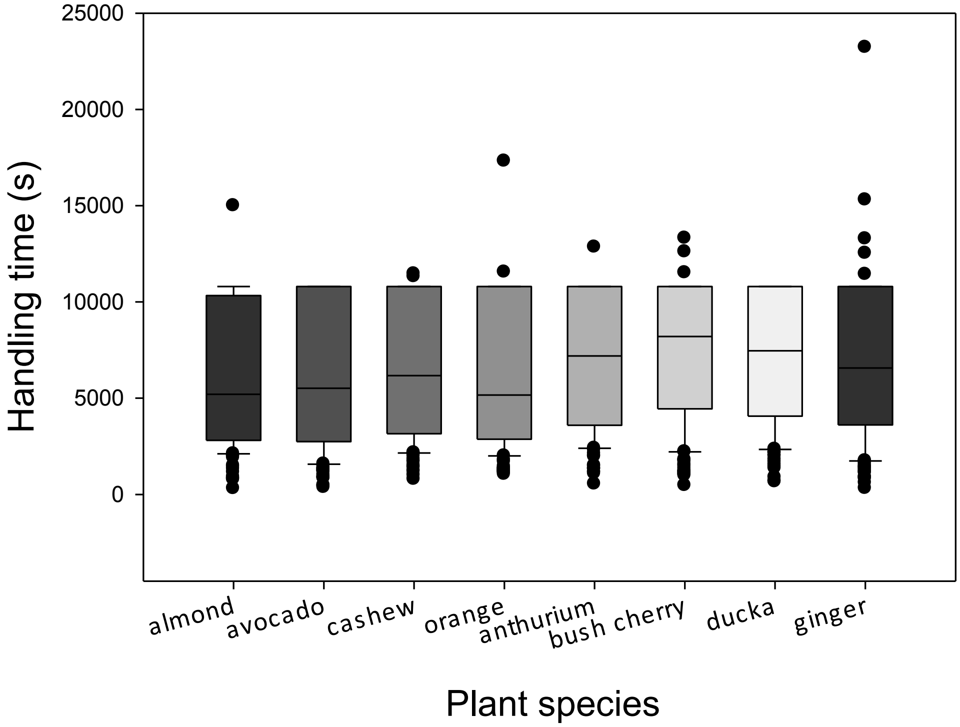
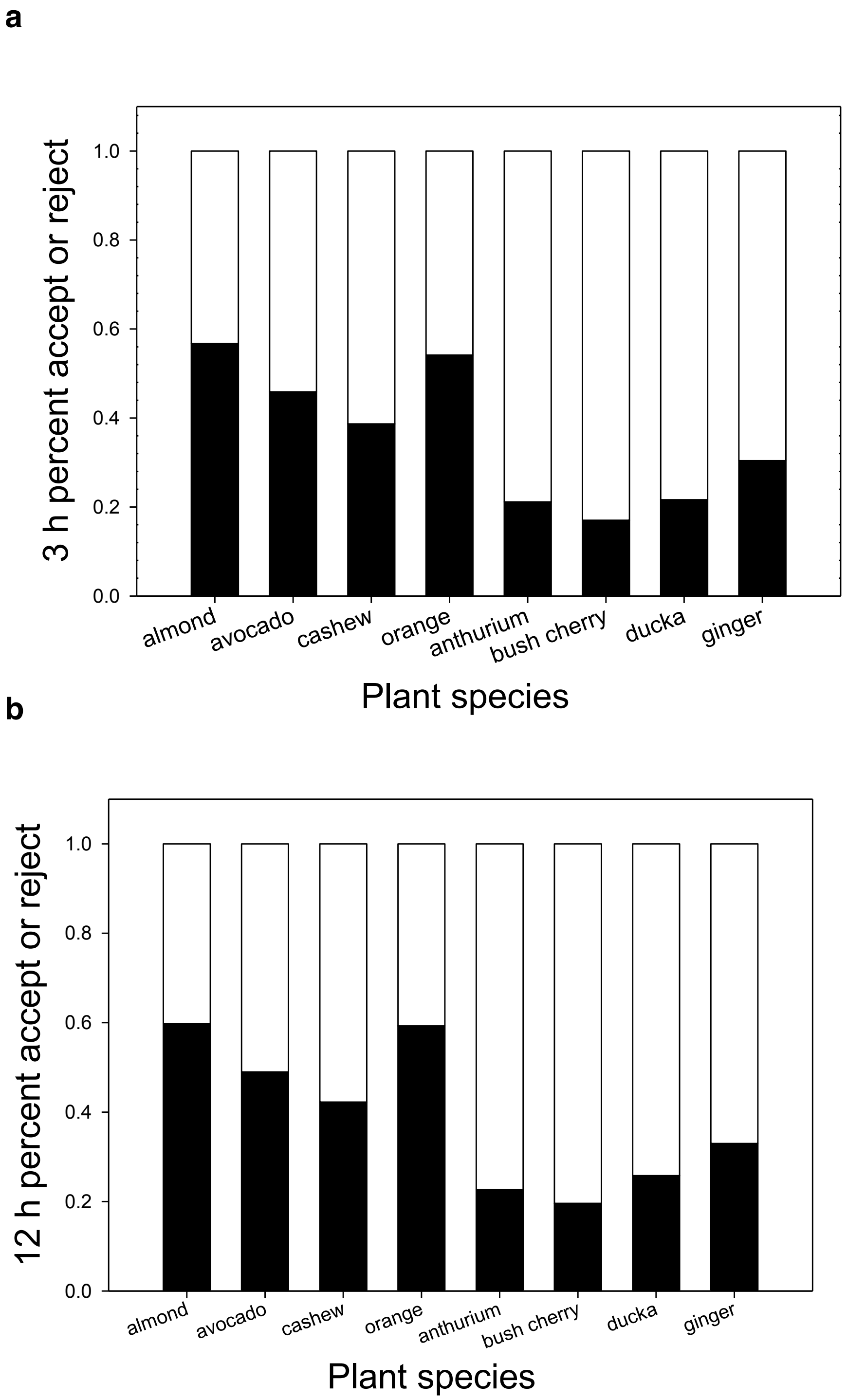


Figure 2. Median handling times (horizontal lines in the boxes) for leaf treatments presented during smorgasbord trials of sampled colonies. The bottoms of the boxes are the 25th while the tops are 75th percentiles respectively. Lower whiskers represent the 10th and upper whiskers 90th percentiles. The closed circles represent extreme values.



3a. Ants accepted orange and almond leaves significantly more often than leaves of other plants up to 3 h after the smorgasbord trials; **b.** Ants accepted orange and almond leaves significantly more often up to 12 h after trials. Black bars represent the percentages of time leaf treatments were accepted and white bars represent percentages of rejections.

Table 1. Non-polar compounds found in sampled leaves of forest and human cultivated species used in the randomized smorgasbord.

Compound Type	west indian almond	avocado pear	cashew	orange	anthurium	bush cherry	ducka	ginger
1R-.alpha.-pinene ($C_{10} H_{16}$)			×				×	
ß-pinene ($C_{10} H_{16}$)				×			×	×
n-Decanoic acid ($C_{10}H_{20}O_2$)							×	
8, 11, 14-Eicosatrienoic acid, (Z,Z,Z)- ($C_{20}H_{34}O_2$)							×	
Limonene ($C_{10}H_{16}$)				×				
11-hexadecenal ($C_{16}H_{28}O$)								×
1-Heptatriacotanol ($C_{37}H_{76}O$)					×			

Discussion

My goal was to determine whether leaf-cutter ants prefer cultivated plants over forest species. I did not find support for P_1 because preferred leaves in the randomized smorgasbord were indicated by shorter handling times, but not discovery times. Sample preference was defined using P_2 such that preferred leaves offered in a randomized smorgasbord were accepted more often than rejected. *Atta cephalotes* preferred leaves of cultivated species, especially orange and almond, over leaves of forest species-thus supporting P_3 that crop leaves would be preferred. However, P_4 was not supported as several preferred leaf samples contained volatiles.

Burton & Holden (2012) suggested that *A. cephalotes* at Karanambu Trust, Guyana preferred crop leaves as these have fewer harmful plant metabolites. Preference order with increasing handling time was cassava (*Manihot esculenta*), orange *(C. sinensis),* avocado pear (*P. americana*), lemon (*C. limon*), lime (*C. aurantifolia*), grapefruit (*C. paradisi*), mango (*Mangifera indica*), and jamoon (*Syzygium cumini*; Burton & Holden 2012). However, in a reciprocal study at Karanambu Trust ants did not abandon cultivated leaves they were transporting for offered whole native plant leaves, and leaf preference could not be discerned by handling time. While *A. cephalotes* at Karanambu Trust and CEIBA Biological Center preferred crop leaves over forest leaves, there was preference variation among ants at CEIBA. Preliminary research at CEIBA indicated variation among colonies for discovery and handling times because colonies processed smorgasbord samples at different rates (Karslake 2014). Furthermore, there was leaf preference variation because ants at some colonies took only crop leaves, but other colonies took both crop leaves and forest leaves (Karslake 2014).

Leaf preferences variation can be explained by the conditioning of leaf-cutter ant colonies to acceptable plants as well as learned avoidance of unpalatable types (Howard et al. 1996; Saverschek et al. 2010). *Atta colombica* presented with experimental vegetation patches containing *Aphelandra golfodulcensis* and *Caryocar costaricense* exhibited more recruitment behavior after encountering a patch with familiar plants (Howard et al. 1996). However, when a scout returned from a mixed patch recruited ants harvested any plant on the foraging trail regardless of the species carried home by the scout. While naïve colonies accepted both *C. costaricense* and *A. golfodulcensis* there was delayed rejection of *A. golfodulcensis* after 24 h even though both plants were acceptable to other colonies (Howard et al. 1996).

Similar delayed rejection of leaf samples containing fungicide were demonstrated by *Acromymex lundi* (Herz et al. 2008). To test how robust learning and memory of unpalatable plants is, previously acceptable leaves were inundated with a fungicide (cycloheximide), and foraging decisions of *Atta colombica* were observed for several months (Saverschek et al. 2010). After two days leaf-cutter ants learned to avoid this plant, and it was 18 weeks before workers harvested it again. This indicated long-term avoidance (Saverschek et al. 2010). Therefore, harvesting and avoidance behavior of ants is dependent on workers' previous experience, and this adaptation is essential in an environment where leaf availability and quality vary throughout the year (Saverschek et al. 2010). This could explain observed delayed rejection at CEIBA where *A. cephalotes* initially accepted most leaf samples offered in the randomized smorgasbord, but later some colonies learned to avoid l species (Karslake 2014).

Despite colony preference variation, leaf-cutter ants are a serious threat to agriculture, as single ant colonies are known to devastate small subsistence farms (Hölldobler & Wilson 1990). Efforts to control these pest populations have included digging up, flooding, gassing, and exploding known nests. Other methods involve placing protective plastic or metal bands around tree trunks and concealing cultivars with grass skirts (Cherrett 1986; Vilela 1986). However, toxic baits have yielded the most success (Vilela 1986), and at CEIBA local farmers often packed poison baits into ant nest entrances. Sometimes to avoid conflicts with leaf-cutter ants, farmers also plant crops, such as pineapples (*Ananas comosus*) which foraging ants mostly avoid (Cherrett 1986). Regardless of efforts to control leaf-cutter ant populations, they still cause considerable economic costs to both plantations and small farms (Hölldobler & Wilson 1990; Vilela 1986). With the increased conversions of tropical forests to farm lands, understanding leaf-cutter ant foraging behavior and plant species preferences may help farmers to minimize conflicts with leaf-cutter ants over depredations of crops (Leal et al. 2014).

In conclusion, I found support for the hypothesis that, leaf-cutter ants already transporting leaves preferred crop leaves over native forest plants. However, leaf-cutters mostly preferred leaves of orange and West Indian almond trees, but *C. sinensis* contained just as many volatiles as some native forest plants. Therefore, other factors define leaf quality, and leaf-cutter ants can learn what plants are palatable, and what plants to avoid. This study was limited because I only sampled eight plant species. Future studies should more thoroughly test the aforementioned hypothesis by including more forest and crop species in randomized smorgasbord tests, and design a statistical model to accommodate foraging attributes such as delayed rejection, learning, and presence of volatiles.

Literature Cited

AZAM, M., Q. JIANG, B. ZHANG, C. XU, & K. CHEN. 2013. Citrus leaf volatiles as affected by developmental stage and genetic type. *International Journal of Molecular Sciences* 14: 17744-17766.

BOURNE, G. R., & C. BOURNE. The biological station and its programs. Pp. 43-45 in Bourne, G. R. & Bourne, C. (eds.). *The CEIBA Reader*. Yerfdog Publishing, St. Louis.

BURTON, B. & F. HOLDEN. 2012. Smorgasbord trials of leaf species preferences by the leaf-cutter ant, *Atta cephalotes*. Abstracts of URS, University of Missouri-St. Louis, St. Louis.

CHAVARRIA PIZARRO, L., H. F MCCREERY, S. P. LAWSON, M. E. WINSTON & S. O'DONNELL. 2012. Sodium-specific foraging by leafcutter ant workers (*Atta cephalotes*, Hymenoptera: Formicidae). *Ecological Entomology* 37: 435-438.

CHEN, Y. H., R. GOLS & B. BENREY. 2015. Crop domestication and its impact on naturally selected trophic interactions. *Annual Review of Entomology* 7: 35-58.

CHERRETT, J. M. 1986. History of the leaf-cutting ant problem Pp. 10-17 in Lofgren, C. S. & Vander Meer, R. K. (eds*.). Fire Ants and Leaf-Cutting Ants.* Westview Press, Boulder.

COBLENTZ, K. E. & S. A. VAN BAEL. 2013. Field colonies of leaf-cutting ants select plant materials containing low abundances of endophytic fungi. *Ecosphere* 4: 1-10.

EL AMINE DIB, M., N. DJABOU, H. ALLALI, J. PAOLINI, B. TABTI, J. COSTA & A. MUSELLI. 2015. Chemical composition of essential oils and hydrosol extracts of and assessment of its antioxidant activity. *Journal of herbs, spices & medicinal plants* 21: 23-37.

HERZ, H., B. HÖLLDOBLER & F. ROCES. 2008. Delayed rejection in a leaf-cutting ant after foraging on plants unsuitable for the symbiotic fungus. *Behavioural Ecology: E1-E8* Advance Access published February 28, 2008.

HÖLLDOBLER, B. & E. O. WILSON. 1990. The fungus growers. Pp. 596-608 in Hölldobler, B. & Wilson, E. O. (eds.). *The Ants*. Harvard University Press, Cambridge.

HOWARD, J. J. 1987. Leafcutting ant diet selection: the role of nutrients, water, and secondary chemistry. *Ecology* 68: 503-515.

HOWARD, J. J., M. LAWRENCE HENNEMAN, G. CRONIN, J. A. FOX & G. HORMIGA. 1996. Conditioning of scouts and recruits during foraging by a leaf-cutting ant, *Atta colombica. Animal Behaviour* 52: 299-306.

HUBBELL, S. P., D. F. WIEMER & A. ADEJARE. 1983. An antifungal terpenoid defends a Neotropical tree *(Hymanaea)* against attack by fungus-growing ants *(Atta)*. *Oecologia* 60: 321-327.

HUBBELL, S. P., J. J. HOWARD & D. F. WIEMER. 1984. Chemical leaf repellency to an attine ant: Seasonal distribution among potential host plant species. *Ecology* 65: 1067-1076.

KARSLAKE, E. B. 2014. Do leaf-cutter ants (*Atta cephalotes*) exhibit crop leaf preferences when already transporting leaves? Animal Behavior Symposium, Princeton.

KAMTHAN, A., M. KAMTHAN, N. CHAKRABORTY, S. DATTA. 2012. A simple protocol for extraction, derivatization, and analysis of tomato leaf and fruit lipophilic metabolites using GC-MS. *Protocol Exchange.* Published online 13 December 2012.

LEAL, I. R., R. Wirth & M. Tabarelli. 2014. The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified neotropical forests. *Biotropica* 46: 516–528.

MARTIN, M.M. 1970. The biochemical basis of the fungus-attine ant symbiosis. *Science* 169: 16-20.

NICHOLS-ORIANS, C. M. 1991. Environmentally induced differences in plant traits: consequences for susceptibility to a leaf-cutter ant. *Ecological* 72: 1609-1623.

NICHOLS-ORIANS, C. M. & J. C. SCHULTZ. 1989. Leaf toughness affects leaf harvesting by the leaf-cutter ant, *Atta cephlotes* (L.) (Hymenoptera: Formicidae). *Biotropica* 21: 80-83.

PERKS, P. & L. MOORE. 2014. Leaf-cutter ants, *Atta cephalotes*, already transporting crop leaves tend to ignore forest plant leaves in smorgasbord trials. Abstracts of URS, 2 May 2014, University of Missouri-St. Louis, St. Louis.

SHANG, Y., Y. MA, Y. ZHOU, H. ZHANG, L. DUAN, H. CHEN, J. ZENG, Q. ZHOU, S. WANG, W. GU, M. LIU, J. REN, X. GU, S. ZHANG, Y. WANG, K. YASUKAWA, H. J. BOUWMEESTER, X. QJ, Z. ZHANG, W. J. LUCAS, S. HUANG. 2014. Biosynthesis, regulation, and domestication of bitterness in cucumber. *Science* 346: 1084-1088.

SAVERSCHEK, N., H. HERZ, M. WAGNER, & F. ROCES. 2010. Avoiding plants unsuitable for the symbiotic fungus: learning and long term memory in leaf-cutting ants. *Animal Behaviour* 79: 689-698.

SEAL, N. J. 2006. Self-organization and the superorganism: functional ecology of the obligate mutualism between a fungus gardening ant and its symbiont fungus. *Electronic Thesis and Dissertations.* Florida State University, Tallahassee. Paper 289.

SCHEINER, S. M. & J. GUREVITCH. 2001. Repeated-measures analysis. Pp. 215 in. Scheiner, S.M & Gurevitch, J. (eds). Second edition. *Design and Analysis of Ecological Experiments*. Oxford University Press, Oxford.

VILELA, E. F. 1986. Status of leaf-cutting ant control in forest plantations in Brazil. Pp. 399-408 in Lofgren, C. S. & Vander Meer, R. K. (eds*). Fire Ants and Leaf-Cutting Ants.* Westview Press, Boulder.

WALLER, D. A. 1986. The foraging ecology of *Atta texana* in Texas. Pp. 159-171. in Lofgren, C. S. and Vander Meer, R. K. (eds.). *Fire Ants and Leaf-Cutting Ants*. Westview Press, Boulder.

WHITLOCK, W. C. & D. SCHLUTER. 2009. the X^2 contingency test. Pp. 213-219 in Whitlock, W. C. & Shluter, D. (eds). *The Analysis of Biological Data*. Roberts and Company Publishers, Greenwood Village.

WIRTH, R., H. HERZ, R. J. RYEL, W. BEYSCHLAG & B. HÖLLDOBLER. 2003. Herbivory of leaf-cutter ants: a case study on *Atta colombica* in the tropical rainforest of Panama. *Ecological Studies* 164.

Chapter 4

Leaf-cutter ant (*Atta cephalotes*) plant defoliation strategies do not quite match central place foraging theory

Elizabeth B. Karslake

Abstract: Leaf-cutter ants (*Atta cephalotes*) are tractable organisms for testing central place foraging models because foragers leave their nest and return with plant fragments. These models predict that animals optimize food acquisition by adjusting their foraging behavior to match the context. Thus, I tested the hypothesis that foraging behavior of *Atta cephalotes* is affected by distances at CEIBA Biological Center, Guyana. This generated four predictions: P_1, to maximize load delivery there are fewer recruited and returning foragers when foraging close to the nest, and more foragers when harvesting leaf fragments farther away; P_2 returning foragers carry heavier, longer, and thicker plant parts closer to the nest, and lighter, smaller, and thinner loads from farther away; P_3 ants make longer trips to harvest cultivated plants because of their higher quality (fewer plant volatiles); and P_4 there is a positive correlation among ant length and plant load mass, length, and thickness as longer ants are documented to carry longer, thicker, and heavier leaf fragments (Wirth et al. 2003). Results partially matched P_4 predictions because ant length had a significant positive linear relationship with both leaf fragment length and thickness. However, weak support was found for the other predictions because unmeasured variables can affect optimality. For example, other studies show that diet selection may include flowers and fruits that are more easily digested by the ants' cultivated fungi, and adjusting the numbers and cast types of recruits reduces congestion in the nest.

Introduction

In optimality models, foragers are expected to forage at patches that maximize energetic intake as well as select food items that optimize their performance and reduce energetic costs (Orians 1980). Therefore, upon leaving their nest a central place forager makes "decisions" about which patch to forage in and how heavy a load to carry home (Dornhaus et al. 2006; Olsson et al. 2008; Olsson & Bolin 2014). There is a proposed tradeoff between travel distance from the central place and acceptance of patch types (Olsson et al. 2014). Individuals who travel farther should selectively forage at higher quality sites, but animals that stay closer must forage at both higher and lower quality patches (Olsson et al. 2014). The appropriate optimality model for organisms obligated to leave from a central place such as nesting birds, hymenoptera, wood rats (*Neotoma floridana*), and human (*Homo sapiens*) hunter-gather populations in search of food or other items is the central place foraging (CPF) model (Dornhaus et al. 2006; Kacelnik & Cuthill 1990; McGinley 1984; Starkovich 2015; Orians 1980). Therefore, CPF theory expects foraging decisions to be affected by 1) travel distances from a central place to foraging patches, 2) handling cost of each load, 3) recruitment rate for social foragers (such as leaf-cutter ants), and 4) quality of items to be harvested (Dornhaus et al. 2006; Olsson et al. 2014; Orians 1980).

Leaf-cutter ants are central place foragers and tractable organisms for CPF model tests (Burd 1996; Burd & Howard 2005; Dornhaus et al. 2006; Kacelnik 1993; Roces & Nunez 1993). The rate at which foragers collect leaves may have evolved to match the slower leaf processing rate in nests at fungus gardens so that smaller leaf loads are favored (Burd & Howard 2005; Wirth et al. 2003). Captive colonies of *A. colombica* demonstrate that the time it takes to process leaf tissue into fungus substrate is longer than foraging roundtrips. Therefore, foragers select optimal load sizes to minimize costs to workers and maximize fungus growth (Burd & Howard 2005). The information transfer hypothesis additionally predicts sub-maximial load delivery by leaf-cutter ants as this facilitates information exchange at the nest (Dornhaus et al. 2006; Kacelnik 1993; Roces & Nunez 1993). In Roces & Nunez's (1993) experiment 1, ants harvested smaller fragments of 10% sucrose solution soaked parafilm and returned to the nest more quickly. Likewise, when harvesting from 1% sucrose solution soaked parafilm ants cut larger and heavier pieces and returned to the nest with slower velocity (Roces & Nunez 1993). In experiment 2, ants again returned more quickly with 10% sucrose soaked filter paper than with 1% sucrose soaked filter paper weighing the same mass. Therefore, when returning with a better quality item ants cut lighter loads and ran with a higher velocity (Kacelnik 1993). Correspondingly, scouts are more likely to display recruitment behavior when returning from a high quality foraging patch, and foragers continually maintain trails leading to good quality vegetation patches (Jaffé & Howse 1979; Wirth et al. 2003).

Foraging leaf-cutter ants encounter many plant species at various distances from the nest, but not all plants are palatable (Dornhaus et al. 2006). Cultivated species are considered high quality vegetation because at CEIBA Biological Center, Guyana leaf-cutter ants accepted leaves of cultivated plants significantly more often than forest species during a randomized smorgasbord. Thus, my goal for this thesis chapter was to determine whether *Atta cephalotes* forage optimally according to CPF theory. I posited that foraging behavior of *A. cephalotes* is affected by distance as expected by some CPF models (Orians 1980). Therefore if distance affects optimal choices ants make about recruitment rate, how heavy of a load to carry back to the nest, and where to forage then the following predictions are realized: P_1 to maximize loads there are fewer recruited and returning foragers when foraging close to the nest, but more recruited and returning foragers farther away (Dornhaus et al. 2006); P_2 returning foragers carry heavier, longer, and thicker plant parts closer to the nest and lighter, smaller, and thinner loads farther away (Olsson et al. 2014); P_3 ants make longer trips to harvest cultivated plants because of their higher quality (fewer plant volatiles); and P_4 there is a positive correlation amongst ant length and plant load mass, length, and thickness as longer ants are documented to carry longer, thicker, and heavier leaf fragments (Wirth et al. 2003).

Methods

Observation of foraging behavior: I observed foraging behavior of *A. cephalotes* at 18 colonies at CEIBA Biological Center from 17 June to 5 July 2014. Leaf-cutter ants were active at night and constructed conspicuous foraging trails. These allowed me to find chosen plants by following recruited ants until foraging trails ended where workers were cutting leaves (Karslake 2014). Here, I measured the distance (m) along the ant's foraging trail to the chosen vegetation patch, and I noted whether the patch contained cultivated or forest species. Then, to look for correlations among worker ant size and characteristics of the harvested plant fragment I randomly selected 20 individuals from each colony. I measured worker length (mm) as well as the harvested plant part length (mm), thickness (mm), and mass (g). Lastly, for each nest site I tallied the number of recruited ants heading along the foraging trail towards the chosen foraging patch for 1 minute (recruitment rate/minute). I also determined returning forager rates/minute, and collected 25 plant particles such as a leaf, flower, or seed from returning foragers (Fig. 1). So overall for each nest, I determined five recruitment and five returning forager rates.

Statistical Analysis: To test P_1 association of distance from the nest (dependent variable) with independent variables, recruited and returning forager counts, I used a multiple regression (Neter & Wasserman 1974). Graph pad-Instat produced a regression line ($\boldsymbol{y}_{\mathbf{i}} = \beta_0 + \beta_{1}x_{i1} + \beta_2 x_{i2} + \ldots \beta_p x_{ip} + E$; β_p parameter for each x_i explanatory variable, E is the error term; Neter & Wasserman 1974). A nonparametric Spearman's rank correlation in Graphpad Instat was used to correlate P_2 distance from a foraging patch (dependent variable) with plant fragment length, thickness, and mass (independent variables; Sokal and Rohlf 1969). A positive Spearman sign indicates that the dependent variable increases with the i_n independent variable, but a negative sign indicates that the dependent variable decreases with i_n. Then, with Graph pad Instat I compared the P_3 mean distance (m) between nest entrance and native plant patches to mixed species patch distance (m) using an un-paired t-test. Data followed Gaussian distributions as indicated by the Kolmogorov and Smirnov method (Whitlock & Schluter 2009). Lastly, to test P_4 I used multiple linear regression to associate worker ant length, as the dependent variable, with the following independent variables (plant fragment mass, length, and thickness; Neter & Wasserman 1974). Data passed the Shapiro-Wilk Normality test, and I used SigmaPlot 11 to produce the population regression line (Neter & Wasserman 1974). SigmaPlot 11 used the least-squares model to determine the best-fit line for observed data, and the Analysis of Variance to describe deviation from expected (Neter & Wasserman 1974).

Figure 1. A sampling of 25 plant fragments obtained from actively foraging *A. cephalotes* during a night of observation. Plant fragments were cut from leaves, flowers, and stem of native tree and pioneer plant species.

Results

Travel distances from vegetation patches to nests were not explained by either recruitment counts or returning forager counts as there was a very weak fit ($R^2 = 0.03$, $p = 0.31$; Fig. 2) to the best fit line (distance = 10.24 + 0.11[forager] - 0.02 [recruitment]). There was also no association among distances from foraging patches to leaf mass ($r_s = 0.18$, $p = 0.025$, $n = 160$), length ($r_s = -0.18$, $p = 0.025$, $n = 160$), or thickness ($r_s = -0.020$, $p = 0.21$, $n = 160$; Fig. 3). Ants traveled a mean distance of 11.83 m to vegetation patches with only native plants and a mean distance of 10.22 m to mixed patches of forest and cultivated species. However, this difference was not significant ($t_{16} = 0.62$, $p = 0.55$). Worker ant length was mediated by leaf characteristics ($R^2 = 0.30$, $n = 360$; Fig. 4), and produced the best-fit-line (worker length = 6.21 – [0.07 x leaf mass] + [0.089 x leaf length] + [0.39 x leaf thickness]). This linear relationship was predicted by combination of leaf length ($p < 0.001$) and thickness ($p < 0.001$), but not leaf mass ($p = 0.94$).

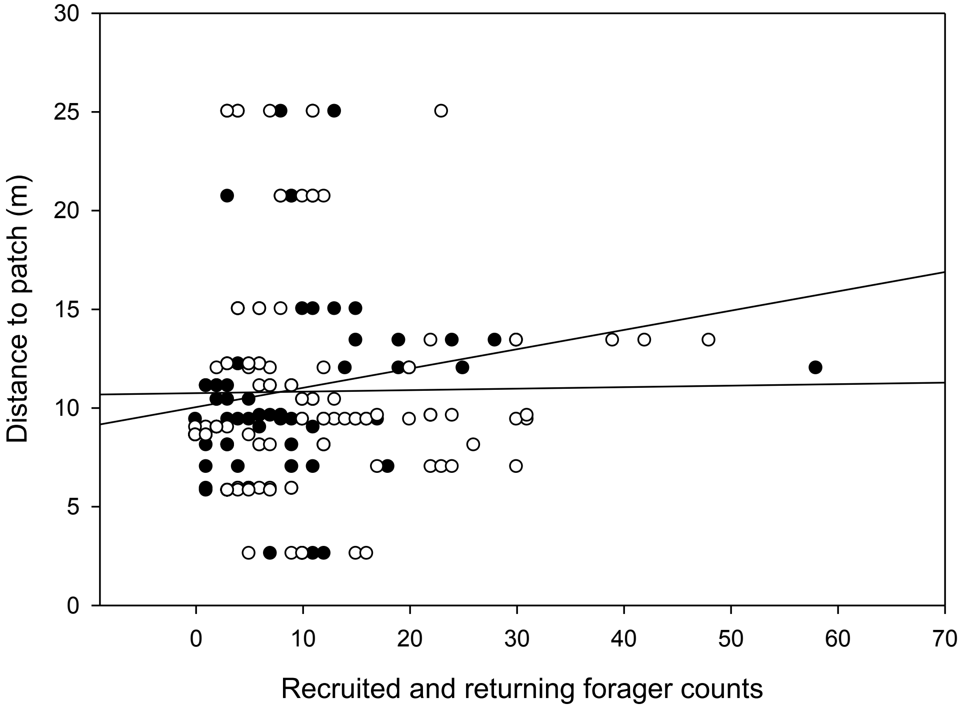


Figure 2. There was no significant associations, between recruited foragers counts (open circles) and travel distance from the nest or between returning forager counts (closed circles) and distance from the nest. The following regression line was produced (travel distance = 10.239 + 0.1060 [returning forager counts] - 0.02185 [recruited forager counts]).

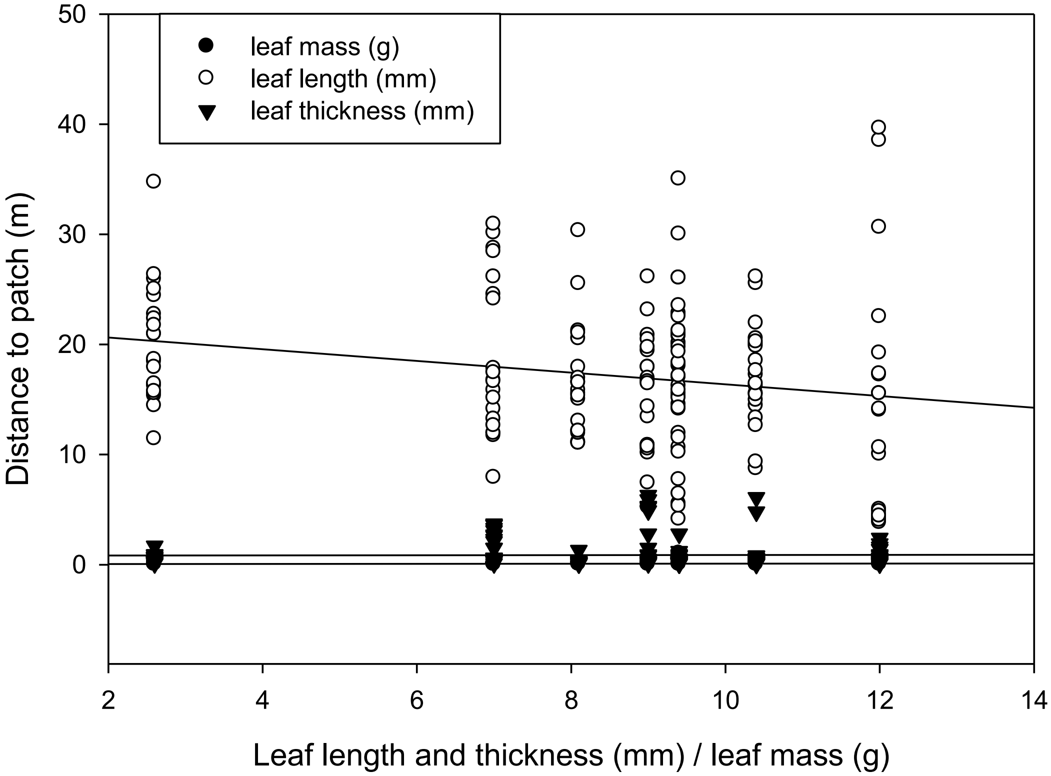


Figure 3. Travel distance from a patch was not predicted by plant fragment length, thickness, or mass.

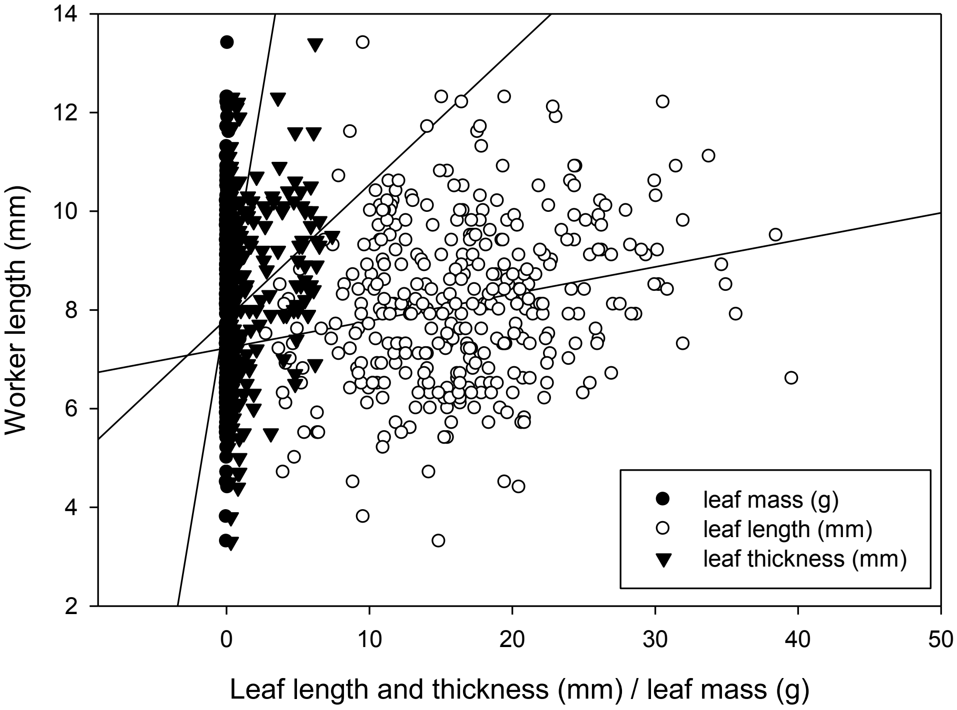


Figure 4. There were significant relationships between leaf fragment length and worker ant length as well as between fragment thickness and ant length. However, there was no significance between fragment mass and ant length.

Discussion

My goal was to determine whether leaf-cutter ants foraged optimally according to CPF theory. I did not find support for P_1 as returning and recruited foragers counts did not increase with travel distance. Likewise, there was no correlation of travel distance from the nest with load size even though P_2 expected foragers to carry smaller, thinner, and lighter fragments at a greater distance. Preference for patches with cultivated plants, as expected by P_3, was not associated with travel distance. I did observe the expected P_4 correlation of forager ant size with plant fragment length and thickness as larger returning foragers carried larger and thicker plant fragments (Burd 1996; Hunt & Nalepa 1994; Kincade 2015). However, load size restricts ants of all sizes when carrying heavier loads up vertical slopes, and in these situations smaller loads may be optimal (Wirth et al. 2003).

Models can be designed for alternative hypotheses, such as the mass dependent costs hypothesis, so that lighter loads are favored by returning foragers (Dornhaus et al. 2006; Kacelnik 1993). Returning *A. colombica* workers often carry loads well below the mass needed to maximize energetic gains predicted by CPF theory (Burd 1996; Burd & Howard 2005). Likewise, foraging honeybees (*Apis melifera*) expend more energy traveling from flower to flower with an increasingly heavy load than returning with a partially full crop (Dornhaus et al. 2006). If foragers expend more energy than gained the net energy gain is negative. Thus, lighter loads are optimal (Dornhaus et al. 2006). The aforementioned information transfer hypothesis similarly predicts sub-maximal load delivery. Returning to the nest with lighter loads and greater velocity allows scouts to facilitate information exchange at the nest (Dornhaus et al. 2006; Kacelnik 1993; Roces

& Nunez 1993). Another population explanation is that leaf-processing rates are slower in the fungi gardens and smaller plant fragments reduce traffic congestion between workers (Burd & Howard 2005). By following simple rules workers and the colony, at large, develop complex and adaptive group foraging behavior (Dornhaus 2012).

Another group adaptive behavior by social central place foragers is 'choosing' foraging patches close to the nest when good quality items are readily available. For example, honey bees (*Apis melifera*) forage closer to the colony in spring than during summer, because summer is the most challenging season (Couvillon et al. 2014). While load delivery methods of honey bees and leaf-cutter ants are very different operations, both social foragers should "not forage at long distances unnecessarily" as this conserves expended energy while searching for food (Couvillon et al. 2014; Dornhaus et al. 2006). Moreover, when harvesting a variety of superior items close the nest there is an expected lower recruitment rate (Dornhaus 2012). *Atta cephalotes* at CEIBA Biological Center had access to many good quality items including cultivated plants as well as flowers, fruits, young leaves, and seeds of native plants. Indeed, the farthest distance ants foraged from the nest was 20.7 m, and during this study workers harvested both cultivated and native plants. Previous studies indicate that *A. cephalotes* in Guyana and other leaf-cutter ant species can travel at least 100 m from the nest in search of vegetation (Cherrett 1968; Wirth et al. 2003). Possibly, in forested areas where cultivated plants are sparse *A. cephalotes* travel farther distances, recruit more workers to cut smaller pieces, and return with lighter loads as predicted by some CPF models (Olsson & Bolin 2014; Orians 1980). Models based off of alternative explanations such as the information transfer (or exchange), mass dependent cost, and worker interactions (term used by Dornhaus et al. 2006) should be used to predict expected behaviors in varying environments.

In conclusion, I did not find support for my hypothesis that foraging behavior of *A. cephalotes* is affected by distance, as ant foraging behavior did not change with increasing travel. Probably *A. cephalotes* in heavily forested habitats, such as Karanambu Trust, Guyana travel greater distances than ants at CEIBA in search of good quality food plants. Thus significant changes in foraging behavior may be observed with increasing travel distance. Future studies should develop a quantitative model that optimizes load delivery to the nest based on differential recruitment of caste sizes. This model should note ant travel distance from the nest, recruitment counts, ant load sizes, returning foragers size, and returning forager velocity. This will distinguish what alternative hypotheses explain foraging behavior of wild *A. cephalotes*. However, it may be as Dornhaus et al. (2006) predicted, that several hypotheses will explain social central place forager behaviors.

Literature Cited

BURD, M. 1996. Foraging Performance by *Atta colombica,* a leaf-cutting ant. *The American Naturalist* 148: 597-612.

BURD, M. & J. J. HOWARD. 2005. Central-place foraging continues beyond the nest entrance: the underground performance of leaf-cutting ants. *Animal Behaviour* 70: 737–744.

CHERRETT, J. M. 1968. The Foraging Behaviour of *Atta cephalotes* L. (Hymenoptera, Formicidae). *Journal of Animal Ecology* 37: 387-403.

COUVILLON, M. J., R. SCHÜRCH & F. L. RATNIEKS. 2014. Waggle Dance Distances as Integrative Indicators of Seasonal Foraging Challenges. *PLOS ONE* 9: E1-8

DORNHAUS, A. 2012. Finding optimal collective strategies using individual-based simulations: colony organization in social insects. *Mathematical and Computer Modelling of Dynamical Systems* 18: 25–37.

DORNHAUS, A., E. J. COLLINS, F.-X. DECHAUME-MONCHARMONT, A. I. HOUSTON, N. R. FRANKS & J. M. MCNAMARA. 2006. Paying for information: partial loads in central place foragers. *Behavioral Ecology and Sociobiology* 61: 151–161.

HUNT, J. H. & C. A. NALEPA. 1994. Nourishment and Evolution in Fungus-growing Ants and Their Fungi. Pp. 309-328 in Hunt, J. H. & Nalepa, C. A. (eds.). *Nourishment & Evolution in Insect Societies*. Westview Press, Boulder.

JAFFÉ, K. & P. E. HOWSE. 1979. The mass recruitment system of the leaf cutting ant *Atta cephalotes* (L.). *Animal Behaviour* 27: 930-939.

KACELNIK, A. 1993. Leaf-cutting ants tease optimal foraging theories. *Trends in Ecology and Evolution* 8: 346-348.

KACELNIK, A. & I. CUTHILL. 1990. Central place foraging in starlings (*Sturnus vulgaris*). II. food allocation to chicks. *Journal of Animal Ecology* 59: 655-674.

KARSLAKE, E. B. 2014. Do leaf-cutter ants (*Atta cephalotes*) exhibit crop leaf preferences when already transporting leaves? Animal Behavior Symposium, Princeton.

KINCADE, I. V. 2015. Leaf-cutter ant cast sizes are related to leaf fragment sizes transported to their nests. Abstracts of URS, University of Missouri-St. Louis, St. Louis.

MCGINLEY, M. A. 1984. Central place foraging for nonfood items: determination of the stick size-value relationship of housing building materials collected by eastern woodrats. *American Society of Naturalists* 123: 841-853.

NETER, J. & W. WASSERMAN. 1974. Multiple Regression. Pp. 214-272 in J. Neter & Wasserman, W. (eds.). *Applied Linear Statistical Models*. Richard D. Irwin, Inc., Homewood, IL.

OLSSON, O. & A. BOLIN. 2014. A model for habitat selection and species distribution derived from central place foraging theory. *Oecologia* 175: 537–548.

OLSSON, O., J. S. BROWN & K. L. HELF. 2008. A guide to central place effects in foraging. *Theoretical Population Biology* 74: 22–33.

ORIANS, G. R. 1980. Tests of central place foraging theory. Pp. 131-140 in Orians, G. R (ed.). *Some Adaptions of Marsh-nesting Blackbirds*. Princeton University Press, Princeton.

ROCES, F. & J. A. NUNEZ. 1993. Information about food quality influences load-size selection in recruited leaf-cutting ants. *Animal Behaviour* 45: 135-143.

STARKOVICH, B. M. 2015. Optimal foraging, dietary change, and site use during the Paleolithic at Klissoura Cave 1 (southern Greece). *Journal of Archaeological Science* 52: 39-55.

SOKAL, R. R. & F. J. ROHLF. 1969. Nonparametric tests for association. Pp. 532-540 in Emerson, R. R. R., Kennedy, D., Park, R. B., Beadle, G. W. & Whitaker, D. M. (eds.) *Biometry: The Principles and Practice of Statistics in Biological Research*. W. H. Freeman and Company, San Francisco.

WHITLOCK, W. C. & D. SCHLUTER. 2009. Two-sample comparison of means. Pp. 337-342 in Whitlock, W. C. & Shluter, D. (eds). *The Analysis of Biological Data*. Roberts and Company Publishers, Greenwood Village.

WIRTH, R., H. HERZ, R. J. RYEL, W. BEYSCHLAG & B. HÖLLDOBLER. 2003. Herbivory of leaf-cutter ants: a case study on *Atta colombica* in the tropical rainforest of Panama. *Ecological Studies* 164.