The Spatial Distribution of Attine Ant Nests on the Firestone Center for Restoration Ecology, Costa Rica

A Thesis Presented
By
Rachel Poutasse

To the Joint Science Department
Of the Claremont Colleges
In partial fulfillment of
The degree of Bachelor of Arts

Senior Thesis in Organismal Biology
December 6th, 2010
Table of Contents:

1. Abstract........................................................................................................3
2. Introduction......................................................................................................4
3. Materials and Methods......................................................................................11
4. Results...........................................................................................................16
5. Discussion........................................................................................................20
6. Literature Cited...............................................................................................24
Abstract:

In neotropical regions, attine (leaf-cutting) ants are common herbivores that act as both ecosystem engineers and a keystone species. They directly impact trees and other vegetation involved in foraging, but also create canopy gaps and modify soil around nest sites, which may in turn affect the type of vegetation that grows near and on nests. The spatial distribution of ant colonies may depend on a variety of factors, including but not limited to foraging preferences, soil type, slope and elevation. It was expected that nest density would be highest in secondary forest over other habitat types as attine ants are known to prefer fast-growing vegetation found in such areas, but would show a dispersed distribution pattern due to the effects of intra- and interspecific competition. *Atta* colonies were located on the Firestone Center for Restoration Ecology in Costa Rica in June and July 2010 using a Trimble Juno ST GPS unit. Nest productivity and age were measured by estimating the nest radius, counting the flow rate (number of leaf fragments/minute) of each active entrance, and following all foraging trails from each nest and recorded the location of the endpoints. In seven weeks, 101 active and 4 inactive attine ant nests were located on the property; *Atta colombica* was by far the most common species. Nests occurred at a density of 1.74 nests per hectare, and were randomly distributed across the property according to the Average Nearest Neighbor test and Ripley’s K test. Nests were frequently found in areas with a moderate slope, but variation was high. Nest density in secondary forest was not significantly higher than in other types of habitat as predicted, which may be due to small patch size and the proximity of different habitat types to each other.
**Introduction:**

For eusocial insects such as ants, choosing an optimal nest site is a long term investment that determines the success and fitness of the colony. Habitat selection may depend on a number of biotic and abiotic factors, including but not limited to the presence of competitors, the abundance of predators and parasites, and resource availability. Although attine (leaf-cutting) ant nests vary in size, *Atta* colonies (which can contain tens of millions of ants) are excellent subjects for spatial studies because they tend to be fairly common, highly visible, and long lived. They are also known to have major impacts on the ecology of the surrounding area. Active nests create a circular vegetation-free gap which greatly increases light availability on the forest floor (Dilcher et al. 2003), while abandoned nests are key recruitment sites for new trees (Garrettson et al. 1998). Attine ant colonies have also been shown to impact the soil around nest sites through nutrient cycling (Farji-Brener & Silva 1995), and the presence of nests can be a driving force behind the types of plants that can be recruited in the area. Due to these impacts on the surrounding area, attine ants have been considered both ecosystem engineers and a keystone species (Correa et al. 2010).

Attine ants have been well studied and are simultaneously an agricultural nightmare and a biologist’s prize example of mutualism. They enjoy a large distribution in North, Central, and South America, and occur from about 40ºN to 44ºS (Weber 1973). In total, the attini consist of 202 species within 12 genera, and have been classified into the more primitive lower attines (*Cyphomyrmex, Mycetarotes, Mycocepurus, Myrmicocrypta, Apterostigma, and Mycetosoritis*), the leaf-cutting higher attines (*Atta* and *Acromyrmex* and the parasitic *Pseudoatta*), and intermediate or transitional attines
(Sericomyrmex, Trachymyrmex, and Mycetophylax) (Leal & Oliveira 2000). All attine ants cultivate and subsist on a fungus monoculture. Of these groups, Atta and Acromyrmex are the most studied, and are well known for their enormous colonies and foraging behavior, which involves cutting and harvesting leaves and other types of vegetation. It is estimated that attine ants are responsible for the herbivory of as much as 12-17% of leaves produced in the tropics (Cherrett 1989). Herbivory by certain groups is also the cause of significant economic losses in agriculture, and management strategies have generally consisted of pesticide application to eliminate ants (Fowler et al. 1989).

In tropical forests it is not unusual to observe long foraging trails of workers bringing cut leaves, flowers, and stems back to the nest. The raw material is further processed by other workers in underground chambers and then used as a substrate to grow the fungus, which serves as a food source for the colony. Specialized workers continually tend to the fungus garden to dispose of various microbes; the fungus itself cannot survive without ant maintenance (Weber 1973).

Attine ants are therefore held in an odd position in which they are required to produce one specific type of fungus to survive, but at the same time must be able to protect it and themselves from attack by other microbes and types of fungi. The symbiotic relationship between ants and fungus is unique and its origins are still the subject of considerable debate. Attine ants and fungus cultivation are thought to have arisen around 50 million years ago as part of a massive adaptive radiation of ant species following the K-T extinction event, although fossil records are poor (Schultz & Brady 2008). Currently there are two models for the evolution of the obligate mutualism: the traditional model, in which ants first incorporated fungi into their diet, then began to
cultivate it, and finally transmitted it between colonies (Weber 1973); and the alternative model, in which fungal spores were first dispersed between colonies by ants, then became part of the ant diet, and then cultivated it (Mueller et al. 2001). Seven major hypotheses have been proposed regarding the original source of the fungal substrate, including stored seeds, nest walls, rotting wood, mycorrhizae, arthropod corpses, and ant feces, all of which support the traditional model; and infrabuccal pellets, which supports the alternative model. Of these hypotheses, nest walls and infrabuccal pellets are considered the most plausible choices for an original substrate (Mueller et al. 2001).

Colonies produce large amounts of waste, which the majority of attine species keep in separate underground chambers to reduce disease transmission. However, in *Atta colombica* and *Atta mexicana*, specialized workers deposit waste in a pile aboveground that is usually located downhill from the nest, most likely to prevent waste from washing into the nest when it rains, therefore reducing the likelihood of contamination. The waste material itself contains large amounts of nitrogen and phosphorous, although the elevated nutrient levels in the soil return to normal within a year after the nest is abandoned (Hudson et al. 2009). Colony size is positively correlated with distance from the nest to the waste pile and negatively correlated with slope, but this mainly occurs in nests with multiple exits for depositing waste (Hart & Ratnieks 2002). Therefore, for *Atta colombica* slope may be one factor that determines nest site selection and success.

Although attine ants are polyphagous, certain plant species seem to be preferred for foraging and ants will often travel considerable distances from the nest for suitable material by utilizing established foraging trails. It has been repeatedly observed that although foraging ants will sample a variety of different plant species, they tend to
selectively focus on only some species for foraging, and the area directly surrounding the nest is typically not stripped of vegetation (Shepherd 1982, Rockwood 1973). This behavior is somewhat odd because it seems likely that energetic costs and predation risk increase the farther an ant travels from the nest. In order for this foraging strategy to be successful, the costs must be outweighed by the benefits of careful selection of plant material and the efficiency of using established trails. Rockwood and Hubbell (1987) found that the use of established foraging trails decreased travel time to and from vegetation sources 4- to 10-fold compared to areas that had not been cleared of litter. According to the palatable forage hypothesis, attine nests may be more common in secondary forest than in primary forest because tree species in secondary forest are in earlier stages of succession. As a consequence, these younger trees are likely to have fewer defenses against herbivory than the older trees found in primary forest. Farji-Brener (2001) found that foraging ants showed a clear preference for leaves from pioneer species over leaves from shade-tolerant plants, which are more likely to occur in older forests where light availability is low. The clearing of mature forest has greatly increased the density of *Atta* and *Acromyrex* in the Amazon Basin and may be allowing atttine ants to expand their range (Vasconcelos & Cherrett 1995). Conversely, as secondary forest ages and is allowed to recover from clearing, nest density drops and colony foraging area increases due to the decline of nearby palatable vegetation (Silva et al. 2009). As deforestation continues throughout Central and South America, an increase in economic losses in agriculture caused by attine ants may be expected.

Dispersal ability is also likely to be an important factor in colony distribution; after mating winged queens will search for suitable habitat to found the colony.
Vasconcelos (1990) found a higher proportion of recently founded *Atta sexdens* nests in forest compared to pasture, and within the forest nest density was higher in areas with treefall gaps, suggesting that the amount of sunlight may be an important factor in habitat choice for foundress queens. Treefall gaps allow for greater amounts of sunlight to enter the canopy, which in turn changes the species composition of plants in that area in favor of less shade tolerant but faster growing species. Jaffe and Villela (1989) attributed the low density (0.045 nests per hectare) of *Atta cephalotes* colonies in undisturbed primary forest in the Amazon Basin to a lack of such clearings.

The issue of scale is obviously relevant in any spatial study. A population that appears to be clustered on a large scale may have a dispersed distribution on a smaller scale. Although no specific mention is made with regards to differences in habitat, a case study of *Atta colombica* and *Atta cephalotes* populations on Barro Colorado Island, Panama revealed that *Atta colombica* nests were concentrated within a 100 ha area with 0.52 colonies/hectare, while for the island as a whole the density dropped to only 0.033 colonies/hectare. According to an average nearest neighbor analysis, this result was significantly different from a random distribution (Wirth et al. 2003). However, the density found by Wirth et al. is low compared to other studies. For example, Rockwood (1973) found a maximum of 2.5 *Atta* colonies per hectare in tropical wet forest in Costa Rica and almost no colonies in tropical dry forest. The nests also appeared to be evenly dispersed within the study area, which the author attributed to competition for space and resources.

Many leaf-cutting ant species are known to exhibit aggressive behavior towards both conspecifics belonging to different colonies as well as ants of different species. *Atta*
*laevigata* workers utilize a particular pheromone near the nest and along foraging trails that serves to stimulate territorial aggression in workers and also to repel intruders (Salzemann & Jaffe 1990). In addition to competition, other species interactions such as predation and parasitism can influence the abundance and distribution of attine ants. Predation by raiding army ants on attine ants (especially of larvae and pupae) has been observed repeatedly in the field (see Swartz 1998 for one example). Additionally, although most species of *Megalomyrmex* ants act as social parasites, members of at least one species are known to usurp attine ant colonies and consume the fungus before moving on to the next colony (Adams et al. 2000). Parasites and pathogens may target the ants or their fungus; the fungus *Escovopsis* directly attacks the cultivated fungus, although ants can reduce infection by utilizing grooming and weeding behaviors (Currie & Stuart 2001).

In this study, the Firestone Center for Restorative Ecology (hereafter referred to as the FCRE or the Firestone Reserve) in Costa Rica was systematically searched for attine ant colonies. The locations of nests on the property were mapped using a Trimble Juno ST GPS unit in order to determine nest density in different habitats and whether nest distribution is clustered, dispersed, or random. A similar project conducted in 2005 located fifty-seven *Atta* nests by searching for ants foraging along the trail systems and without the use of GPS (Wheeler); unfortunately, there is no map showing the locations of the colonies in 2005 for comparison. Furthermore, although the 2005 study does calculate nest density in different habitats on the reserve, it is unclear how the author determined the habitat type and which areas the classifications were applied to.
Much of the reserve was formerly used for cattle pasture and growing bamboo, which as a consequence currently contains a number of different habitats including newer secondary forest, areas of primary forest mainly located along creeks, and bamboo forest on the far west side of the property. In accordance with the palatable foraging hypothesis, it was expected that nest density would be highest in secondary forest, but nests would not be clustered closely together. Previous studies (see Rockwood 1973) have demonstrated a dispersed distribution pattern for *Atta* nests, which may be related to intraspecific aggression between colonies; a similar pattern was expected in this situation. Finally, it was hypothesized that *Atta colombica* nests in particular would tend to be located in areas with a strong slope in order to allow the aboveground waste pile to drain away from the colony during rain.
Methods and Materials:

Study Site

The Firestone Center for Restoration Ecology (FCRE) is property of Pitzer College and is located on the Pacific coast of Costa Rica, adjacent to the Hacienda Baru Reserve. Much of the 150 acre reserve was cleared for cattle farming in the 1950s and 1960s, with efforts made to restore the property to its original state beginning in 1993. Consequently, the FCRE currently consists of a number of different habitats, including older riparian forest along the three major creeks, bamboo plantation and forest along the west end of the property, a small area used as banana plantation on the southwest portion of the property, and newer secondary forest for the majority of the reserve. The areas of the major habitat type were determined based on comparisons of two aerial maps of the reserve from 1972 and 2006 (Figures 1 and 2). The FCRE is also located on a slope, which becomes much steeper as one goes from the east end of the property to the west end.

Locating nests:

Because it can be difficult to thoroughly census a large area of forest, several different techniques have been used to locate and record the density of attine nests. Rockwood (1973) used a combination of searching several 4-hectare quadrats in different habitats for nests, and additionally recorded the number of colonies viewed by walking along a series of transects. In 2005 Wheeler treated existing trails within the Firestone Reserve as 100 m transects, with one analysis only including colonies within 10 m of either side of the transect and the other analysis including colonies up to 50 m away from the trail. With the recent advancements in GPS technology, it is now possible to survey
larger areas more accurately. Although GPS can be an important tool for conducting spatial studies, it does have some disadvantages. The precision of readings depends on satellite reception, which can be diminished in areas with heavy overhead cover or at the bottoms of canyons. However, low satellite reception was not generally a problem in this study, except for a few areas in the northeast corner of the reserve along the creek.

The map of the Firestone reserve was divided into a grid consisting of 100 m² squares. From June 1, 2010 until July 22, 2010 (the beginning of the wet season) the entire reserve was systematically surveyed except for a few areas that were impossible to access safely. Surveying was always conducted between 9:00 in the morning until no later than 3:30 in the afternoon, and was never done in the rain, as most foraging trails are not active during rainstorms. Rainstorms occurred on an almost daily basis, usually beginning mid-afternoon and continuing throughout the evening and night. Within each complete square the search began at one edge or corner and proceeded by walking in a zigzag pattern across the square about four times. A compass and a Trimble Juno ST GPS unit were used to locate the border of the squares and navigate across. The same method was used to cover partial squares with greater than half of the area of a full square (for example B05 or B06). Partial squares with less than half the area of a full square (for example A05 or A06) were covered in conjunction with the nearest full square.

When a trail of ants was discovered foraging along or near the path, the ants were followed back to their nest, which was flagged with biodegradable tape and labeled “LCA” (Leaf Cutting Ant) followed by a number. When data recording at the nest was complete, the search was continued by returning to the location where the first foraging
trail had been found. During the first week the search methods were different because the program needed to navigate on the map had not yet been downloaded to the GPS unit; instead, attine ant trails were searched for along the trail system (nests 1-16). Once a nest was discovered, the same protocol was implemented as stated below for all nests.

**Measuring size, productivity, and foraging radius:**

Nests were considered to be active if there were foraging trails or ants present. At active nests, the presence or absence of a refuse pile was noted; the locally abundant *Atta colombica* disposes of waste in noticeable piles aboveground unlike other species. The number of entrances was recorded the radius of the nest was estimated, and as most nests were roughly circular. Active entrances were defined as those where foraging ants carrying cut leaves were returning. The number of ants returning to the nest with leaf material in a one minute interval was counted at each active entrance (flow rate). Active trails were then followed until the source where the leaves were being taken from was discovered; this included all endpoints for foraging trails that split into multiple smaller trails. Each source was flagged with biodegradable tape labeled “LCA” followed by the nest number and a letter and the location was recorded with the GPS unit in order to determine a crude foraging radius. Collection points in which the ant trail led to a location or nest off the property were also included, while ant nests within the reserve with collection points located off the property were recorded with the GPS unit at the farthest edge of the property.

In order to determine the consistency of the foraging trails and productivity, flow rates were also monitored at all active entrances of two nests (LCA 54 and LCA 13; both *Atta colombica*) located near the dormitory at the east end of the property for twenty-two
days from June 23, 2010 to July 22, 2010 in the morning (before 9:00am), midday
(12:00-5:00), and at night (after 5:00pm) regardless of weather conditions.

Spatial Analysis:

Each day the data on the GPS were downloaded onto Cybertracker to create a
map of the Firestone Reserve depicting the locations of ant nests and collection points.
The final data set was transferred to ArcMap 10 for spatial analysis. All maps were
created using the same program. The Average Nearest Neighbor analysis and Ripley’s k-
function were run on all nests that were found on the reserve to determine whether the
distribution was clumped, random, or evenly distributed. Nest density was calculated as
number of nests per hectare. A Chi Square test was run in SPSS 17 to compare the
relative number of nests in each habitat. The expected nest frequencies were calculated
based on the percent of the reserve occupied by each habitat type. If nests are not
clustered by habitat, then the percent of nests in each habitat should not differ from the
percent of the reserve taken up by each habitat.
Figure 1. Aerial image of the Firestone Reserve in 1972 showing the reserve boundary, major creeks, ponds, and trails.

Figure 2. Aerial image of the Firestone Reserve in 2006 showing the reserve boundary, major creeks, ponds, and trails.
Results:

In seven weeks of surveying, 105 *Atta* nests were found on the Firestone Reserve at a density of 1.74 nests per hectare, including four nests which were considered inactive due to the absence of foraging trails or ant activity. Among the four major vegetation types, nests were most common in secondary forest followed by primary forest, bamboo, and banana (Figure 3); however, nest density was not significantly different between different habitats (Chi Square=5.53, df=3, p=0.137) (Figure 4). Although it was not possible to identify every species, all except thirteen of nests had aboveground refuse piles, which in Costa Rica only occur in *Atta colombica*. The average nest radius was 3.3 m, although there was a considerable range from 0.1 to 12.3 m. The mean straight-line distance of foraging trail endpoints to the nest was 35 m; the longest foraging trail extended more than 180 m away from its colony.

**Figure 3.** Locations of attine ant nests on the Firestone Reserve according to habitat type and ant species as of July 2010.
Figure 4. Attine ant nest density per hectare in different habitat types on the FCRE as of July 2010. Blue = *Atta colombica* (n=87), red = other attine ant species (n=13).

The foraging radius of each colony was calculated\(^1\) as a function of the furthest collection point from the nest. The foraging area of 45 out of 92 nests on the reserve encompassed two or more habitat types. Colonies with foraging radii that extended outside the reserve boundary were considered to utilize multiple habitats.

Figure 5. Frequency of the number of habitat types within the foraging radii of different attine ant nests on the Firestone Reserve as of July 2010 (n=92).

\(^1\) By Kevon White and Warren Roberts.
Average Nearest Neighbor and Ripley’s k-function:

*Atta* nests were randomly distributed on the Firestone Reserve as a whole (Observed Mean Distance/Expected Mean Distance=0.95, p=0.326). Ripley’s k-function also showed an overall random nest distribution (p=0.486).

Slope:

Nests were overall found to be most common on a moderate slope, although there was a considerable range (mean±SD=21.6º±14.3º). Broken down by species, mean nest slope was very similar for *Atta colombica* and other attine ant species, although the sample size of *Atta colombica* nests was much larger than the sample size for other species (*Atta colombica*, mean±SD=22.1±14.3º, n=85; other attine ant species, mean±SD=17.1±14.4º, n=13). The means only include nests that occurred within the boundary of the reserve; there was no slope data available for the two *Atta colombica* nests that were counted outside the boundary due to slightly inaccurate GPS readings.

**Figure 6.** Frequency of attine ant nests on different slopes on the Firestone Reserve by species as of July 2010. Blue = *Atta colombica* (n=85), red = other attine ant species (n=13).
Figure 7. Locations of attine ant nests on the Firestone Reserve as of July 2010 in relation to slope in degrees.
Discussion:

Overall, this study did not identify areas of clustered or dispersed nest distribution within the Firestone Reserve. The results were also not consistent with the palatable foraging hypothesis, which implies that nests should occur more often in secondary forest than primary forest because of better quality foraging material (Figures 3 and 4). There are several possible explanations for this pattern (or lack thereof). The current vegetation map is based off of fairly low resolution aerial images from 1972 and 2006, which do not allow for finer classification of different habitats. For example, the patches of secondary forest on the east end and west end of the reserve varied greatly in terms of composition and quality. With improved resolution and better ability to distinguish differences within habitat types, it may be possible to get a stronger sense of whether nest distribution is truly random. Furthermore, most primary forest on the Firestone Reserve is broken up into multiple small patches, generally located along creeks and the boundaries of the property. Colony density and distribution patterns that appear in large areas of more uniform forest type may be less applicable in this situation. Foraging ants may be able to easily cross over from one vegetation type to the other since there are numerous patches of different habitat in a relatively small area. This could explain the lack of a strong preference for any particular habitat or area of the reserve. For the majority of colonies on the reserve, preliminary results do indicate that the foraging areas of many colonies on the reserve can and often do encompass multiple vegetation types (Figure 5).

The Average Nearest Neighbor analysis and Ripley’s k-function did not show a dispersed nest distribution, which seems to contradict the findings of other studies. This dispersed pattern has usually been attributed to competition and aggression between
colonies (Rockwood 1973). However, Weber (1973) claims that aggressive behavior is not common in attine ants and ants from different colonies are more likely to ignore each other. In the field any kind of conflict between attine ants was rarely witnessed, although it was uncommon to find large nests close together. This does not rule out the possibility of aggressive interactions occurring, but such interactions may be infrequent because of the costs they could potentially incur. In one study, an aggressive encounter between attacking *Atta sexdens* workers and *Acromyrmex crassispinus* workers defending the nest resulted in the complete disruption of foraging by *A. crassispinus*, left the colony vulnerable to predators, and lasted for 17 days (Fowler 1977). Furthermore, it is likely that certain species may display aggressive behavior more than others; in several cases *Atta colombica* nests were situated near nests belonging to different species without obvious problems (Figure 3). Even though the foraging areas of different colonies may overlap, the use of well-established foraging trails could reduce the frequency of encounters. The random nest distribution pattern that emerged on the Firestone Reserve may point to the greater importance of other factors over competition.

With regards to slope, nests overall did occur in areas with at least a moderate slope, though there was a wide range (Figure 6). The mean slope for *Atta colombica* nests was very similar to the mean slope of other species’ nests, although this could be partially attributed to vastly unequal sample sizes. Further analysis is needed to show whether larger nests with multiple waste exits tend to be located on steeper slopes, as was found by Hart and Ratnieks (2003).

Although this analysis provides only a snapshot of a single component of the ecosystem at one point in time, future recensusing of the area may allow us to develop a
better picture of colony stability and turnover rates. The areas of secondary forest at the reserve are relatively new and for the most part low quality. It will be interesting to see whether colony density gradually declines as the forest ages and the abundance of preferred vegetation decreases as found by Silva et al. (2009). It is generally agreed that colonies can last for long periods of time; Wirth et al. (2003) claims that established *Atta* colonies may survive for up to 15 years. However, there are few studies tracking the survival and growth of new nests, which may be short-lived and have lower impacts on the surrounding environment relative to larger established nests. Additionally, because recently established nests are small, they are more likely to be missed while searching.

At the time the survey was conducted, there were not sufficient resources to identify the tree species utilized by the ant foraging trails. It was also not possible to definitively identify ant species apart from relying on the presence or absence of an aboveground refuse pile. The ability to collect such data in similar future projects could be valuable for conducting more in-depth tests of the palatable foraging hypothesis as the forest ages, even though the results of this study did not show a preference for secondary forest as nest sites. It was observed that although there were some nests located within bamboo forest and the banana plantation, these nests tended to be small and located at the edges of the habitat. It was very rare to find foraging ants cutting bamboo or banana plants, which suggests that these plants are either energetically costly to cut, are a poor substrate for fungal growth, or both.

Although attine ant nest density is determine by numerous factors, colonies also play an important role in shaping and altering tropical ecosystems and in agriculture due to their considerable impacts on surrounding vegetation and other aspects of their habitat.
There is a certain level of irony in the fact that forest cleared for agricultural purposes has been linked to increased attine nest density, which in turn results in increased crop herbivory and economic losses. As humans continue to alter the natural environment in the tropics, whether through deforestation or reforestation, there are likely to be further changes in the distribution of attine ants and many other species in years to come.
Acknowledgments:

I am grateful to many more people than I can list here for making this possible. I would like to thank my advisor Dr. McFarlane for his never ending support, background knowledge and advice for this project, my second thesis reader Dr. Purvis-Roberts for her helpful input, and Warren Roberts for his assistance with the GPS equipment, creating maps, and analyzing data. Dr. Thomson and Professor E. Ferree additionally contributed useful advice regarding experimental design and statistical analysis. Finally, I would like to thank the staff at the Firestone Reserve, my project partner Kevon White, and the rest of my Firestone family—Gizelle Pera, Keala Cummings, Adam Hanbury-Brown, and Ben Storlie—for their hard work and all the wonderful memories. We did it!

Literature Cited:


