

Investigating post-harvesting leaf selection filters in leafcutter ants (*Atta cephalotes*)

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ABSTRACT

Atta species leafcutter ants have a symbiotic relationship with fungi. The ants use the fungus as a food source, so it is critical that the fungus is tended properly. To meet the fungus's needs, they have several distinct castes which perform specific functions, and are morphologically distinct. The gardening caste is responsible for assessing the current health and dietary needs of the fungus, and communicating this information to the foraging caste. However, the mechanisms by which the different castes communicate and disseminate information are not well understood. Leafcutter ant colonies can contain millions of individuals, suggesting that the ants have a specialized system for collective decision making. Other types of colony-dwelling ants make decisions by quorum voting, in which individuals vote until a certain threshold is reached. I observed an *Atta cephalotes* colony in Costa Rica to investigate the potential role of quorum voting in post-harvesting leaf selection. I also measured the size of leaf fragments dropped along a transect along the main leafcutter path to determine whether fragments dropped near the colony differed in size from those dropped farther away. I found that leaf rejection usually occurred after several small ants morphologically consistent with the gardening caste physically contacted the fragment and the forager. However, there was no significant trend in fragment size along the transect. These results suggest that the gardening caste has a previously undescribed role in filtering leaf fragments near the colony, but that fragment size is not a factor in near-colony leaf selection.

Atta cephalotes is one species of leafcutter ants, which are a group of 47 species of tropical new-world ants that have obligate mutualisms with fungi (Speight *et al.* 2009). They live in large colonies containing millions of individuals and occupying a radius greater than 80 meters (Rockwood 1976, Moreira *et al.* 2004). Within their extensive colonies, they cultivate a

fungus which is fed to ant larvae (Mueller *et al.* 2001). To promote the growth of the fungus, they harvest leaf fragments from trees and bring them back to the colony for use as fertilizer. The ants can detect and respond to the needs of the fungus by altering the quantity and qualities of the leaves they harvest (North *et al.* 1997). This ant-fungus mutualism is obligatory, because the fungus needs to be fertilized by leaves harvested by the ants, and the ant larvae must consume the fungus.

Leafcutter ants have robust sensory systems for communication with each other and their fungi. They possess sensitive chemical and tactile signaling systems which are important in their farming activities. To tend to their fungi effectively, they respond to chemical cues from the fungus by altering the types of leaves they harvest (North *et al.* 1997). Like many species of ants, they also communicate with conspecifics via chemical communication. They also use tactile cues to communicate with each other, particularly for decision-making and pathfinding purposes (Hager *et al.* 2017).

The mechanisms by which leafcutter ants disseminate information about their fungus's changing needs and subsequently alter their behavior are currently not well understood. The size of their colonies presents a challenge for effective colony-wide behavioral changes. Pratt (2005) found that a different species of colony-dwelling ant, *Temnothorax albipennis*, make decisions that lead to alterations of the behavior of the entire colony through quorum voting, where individual ants "vote" until a threshold number is reached. After the threshold is reached, they rapidly communicate the decision and induce colony-wide behavioral changes. In this study, I assess the role of quorum activity in leaf selection near the colony.

This study focuses on *Atta cephalotes*, a widely distributed neotropical ant species (Corrêa *et al.* 2005). The behavior of the *Atta* family is well characterized; Wilson (1980)

described four castes in *Atta sexdens* that generally perform different tasks and have distinct morphology. In order of increasing size, these castes are gardeners/nurses, within-nest generalists, foragers/excavators, and defenders. Gardeners/nurses specialize in fungus and larval care and ride on leaf fragments during transport. Within-nest generalists perform many functions, including degrading vegetation, disposal of waste, and queen care. Foragers/excavators scout, harvest, and transport vegetation, recruit other foragers, and expand the nest. Defenders protect the nest from threats. Wilson also states that each caste, apart from defenders, can be subdivided further because younger and older ants within each caste perform distinctly different tasks.

At the study site, I noted that there was a significantly greater number of abandoned leaf fragments near the colony entrance, and the number of abandoned fragments decreased dramatically with distance from the colony. This observation was puzzling because the ants often travel hundreds of meters through dense forest to access a specific tree. Transporting these leaves, which may weigh more than eight times as much as the ants themselves, is therefore a significant energy expenditure (Segre & Taylor 2019). Consequently, I proposed that there is an additional selection filter near the colony because it would be unfavorable for an ant to expend this energy if it was capable of filtering leaf fragments prior to transporting the leaf back to the colony. In this study, I assessed the physical characteristics of leaf fragments rejected near the colony entrance vs. along the leafcutter ant trails, to determine if leaf fragments are filtered based on their physical properties. In particular, I examined the average surface area and mass of leaf fragment for at varying distances from the colony, to determine if leaf fragments dropped near the colony differ in size compared to those dropped elsewhere. I also assessed the behavior of ants before, during, and after rejecting a leaf to determine how the ants decide to reject a leaf. Specifically, I examined the role of quorum voting in leaf rejection.

METHODS

Leaf fragment collection. — I collected leaf fragments near an *A. cephalotes* colony from 6-8 January 2024, at Camaquiri Conservation Initiative (CCI) in Limón, Costa Rica ((10° 32' 9" N, -83° 44' 41" W). The *A. cephalotes* colony is located downhill of the laboratory at CCI (Fig.1).

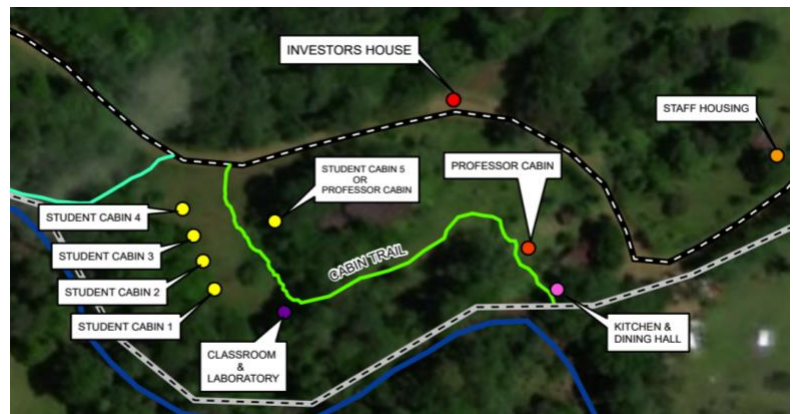


Figure 1: Buildings and Cabin Trail at CCI. The *A. cephalotes* colony was located near the classroom and laboratory (purple dot)

On 6 Jan, I placed markers every meter from the main colony along the major ant path until it diverged and entered the woods. I designated the largest entrance as marker 0. My sampling site ranged from -2 to 23 meters from marker 0, with negative markers being uphill and positive markers being downhill from marker 0. I constructed a 30x90cm quadrat and sampled leaf fragments by placing the quadrat adjacent to a marker and collecting all leaf fragments within the quadrat area. I sampled one third of the quadrats each day, by sampling every third quadrant. Each day I shifted my starting position by one meter. I only collected leaf fragments that were resting loosely on the surface, and which visually appeared to have been cut by the leafcutter ants based on the sharpness and angularity of the cuts. Since this colony was primarily nocturnal based on my observations, I collected all the leaf fragments in the morning so my presence didn't disrupt their activities.

Leaf fragment measurements. — After collection, I brushed any dirt off the leaves with my fingertips. On 7 Jan and 8 Jan, it rained overnight until approximately 9 am and 11 am

respectively, so I waited until the rain had stopped and the ground had dried before collecting the leaf fragments. After cleaning and allowing the leaf fragments to dry, I placed the fragments on a white piece of paper and photographed them alongside a ruler for scale. I also recorded the number of leaf fragments and total mass of leaf fragments from each quadrat. In total, I sampled 1554 leaf fragments.

Leaf fragment analysis. — I manually edited out shadows and used ImageJ to analyze the leaf fragments. I used the ruler in each photo to set the scale. I converted each image to 8-bit using ImageJ and set the threshold based on my judgement to isolate each fragment from the background. I added each leaf individually to the ROI manager and measured the area of each fragment.

I analyzed the leaf fragment data using RStudio. I performed a one-way ANOVA of leaf areas in each quadrant. I calculated the average mass per leaf in each quadrat by dividing the total mass in each quadrat by the number of leaves collected in that quadrat. I also calculated the average mass per surface area, indicative of leaf density or thickness, by multiplying the average leaf area by the number of leaves in a quadrant, and dividing by the total mass of leaves from that quadrat.

Ant behavior videos. — From 5-8 Jan 2024, I recorded videos of ant behavior at marker 0 in ten minute intervals. On 5 Jan, I recorded every half hour from 7:00pm-9:30pm. On 6 Jan, I recorded at 10:30am, 2:00pm, and 8:30pm. On 7 Jan, I recorded one video at 8:30pm. On 8 Jan, I recorded every hour from 5:45pm-10:45pm. I later watched the ant behavior videos and noted instances

where a leaf was dropped. I also described the behavior of the ants prior to and after dropping a leaf. I observed 8 instances of leaves being dropped across six different videos.

RESULTS

NUMBER OF LEAF FRAGMENTS. — The number and total mass of leaf fragments per sampling area decreased exponentially along the sampling line (Fig. 2), supporting my observations that more leaf fragments are dropped near the colony.

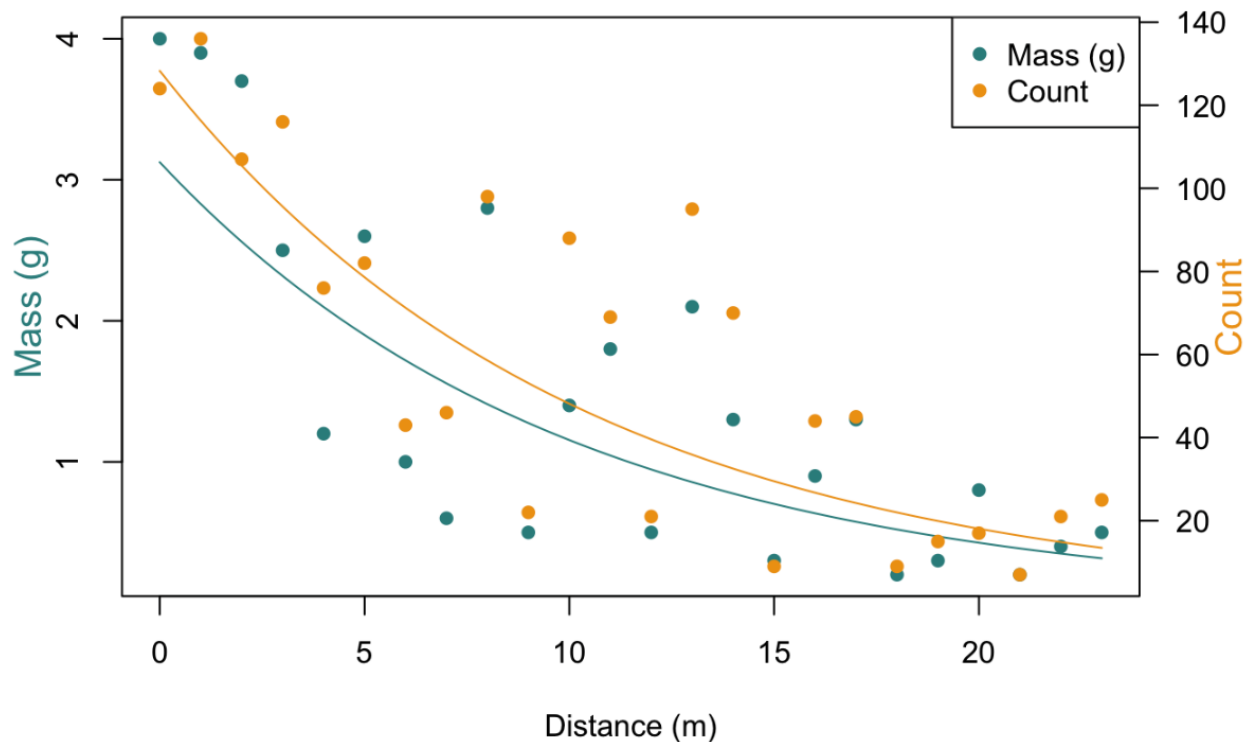


Figure 2: Leaf count and total mass decrease with distance from the colony main entrance, demonstrating that more leaf fragments are rejected close to the colony. R-squared (mass) = 0.57; R-squared (count) = 0.58.

LEAF FRAGMENT AREA. — Leaf area did differ significantly between sampling areas (ANOVA: $F=3.685$, $df=25$, $p>0.0001$). The Tukey post-hoc test found that mean leaf areas were similar between meters -2 through 19, and 21 through 23. Meter 20 was the only sampling area with a

significantly higher mean leaf area. However, although the trend is not significant, mean leaf area seemed to decrease slightly until the middle of the colony (approximately meter 7) before rising again (Fig.3).

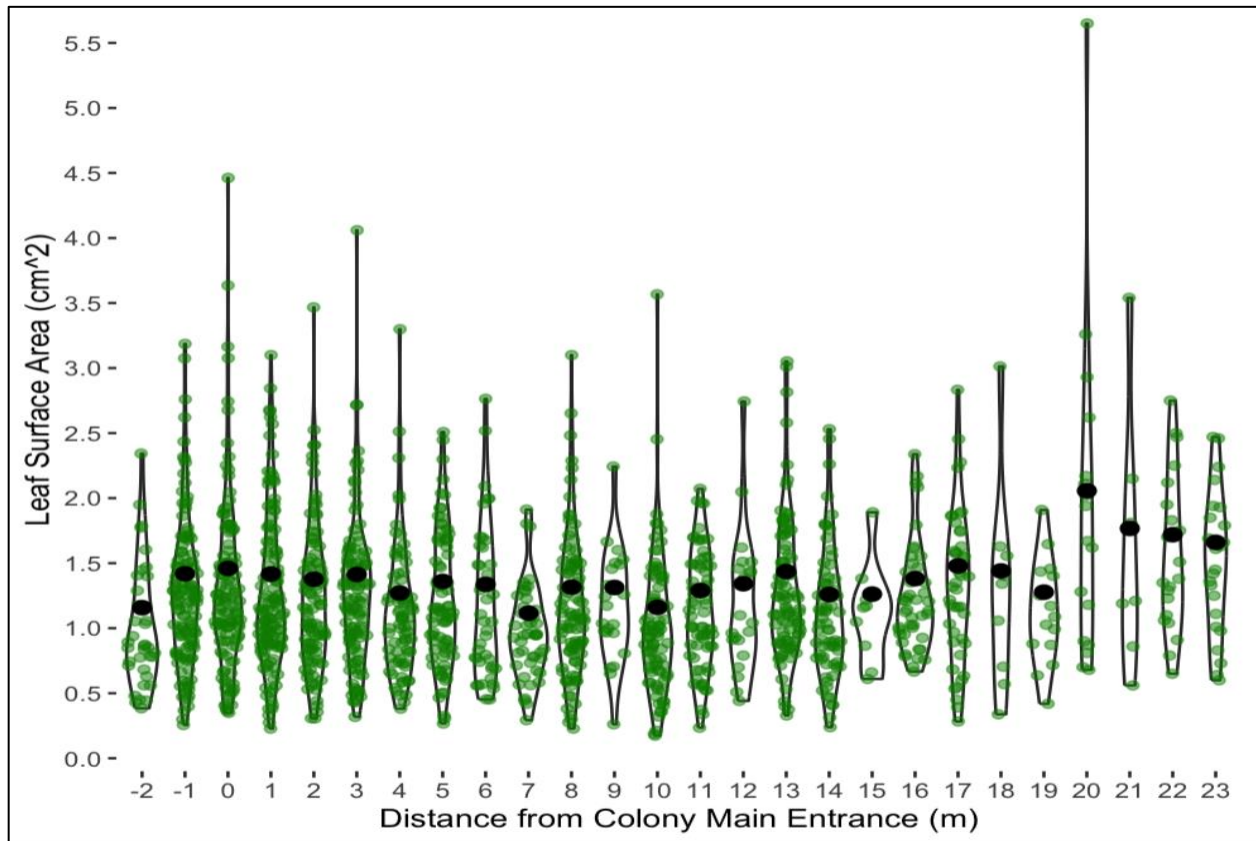


Figure 3: Mean leaf area does not differ significantly between any sampling area, except meter 20 which is significantly greater than the average of meters -2 through 19. Green dots represent the area of individual leaf fragments, and black dots indicate the mean leaf fragment area. Width of the violin plots illustrate the distributions of the data. Leaf size was not a significantly more important factor in leaf selection near vs. far from the colony.

LEAF FRAGMENT DENSITY. — Total mass per quadrat increased exponentially with number of leaves, suggesting that where more leaf fragments were rejected, the fragments were on average thicker or denser (Fig. 4). However, there was no correlation between average mass per area and distance, even though the number and total mass of leaf fragments decreased along the sampling line.

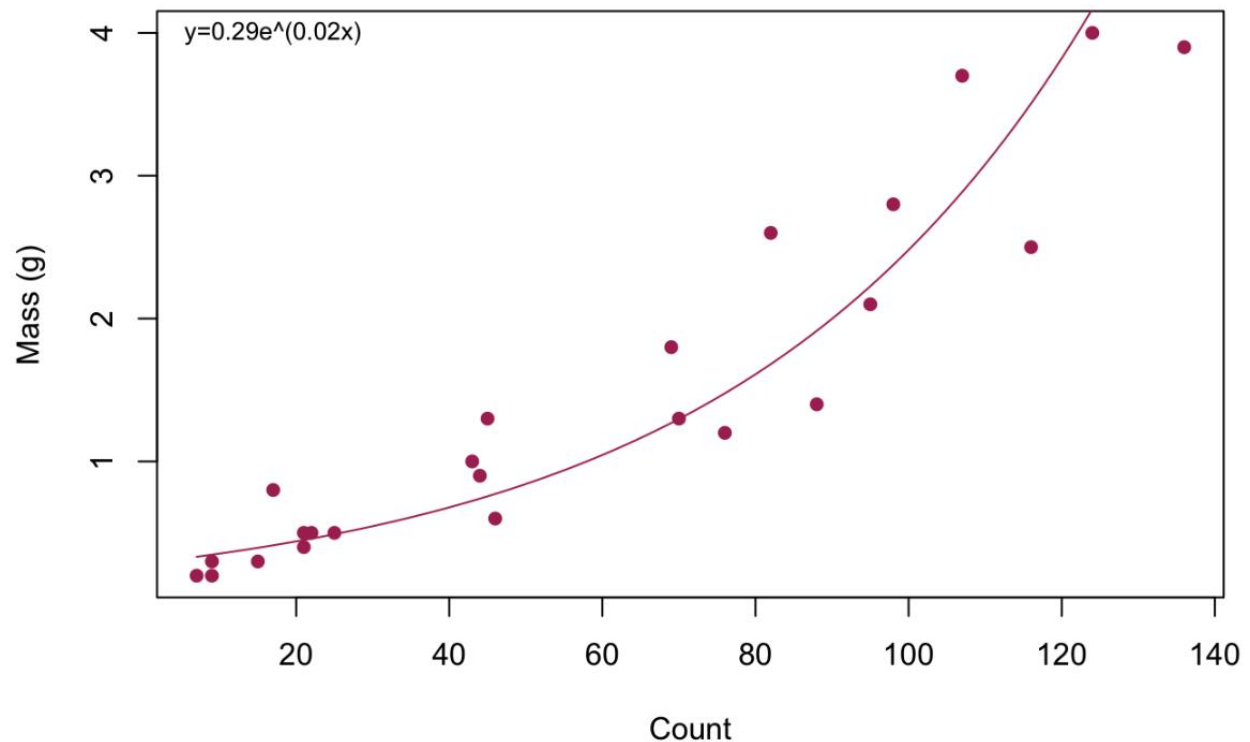


Figure 4: Leaf mass per quadrat increases non-linearly with leaf count, suggesting that where more leaf fragments were dropped, the fragments were on average denser or thicker. R-squared = 0.88.

ANT BEHAVIOR. — Each of the eight observations of leaf rejection occurred between 8:30 and 10:00 PM. Most of the rejection events followed the same behavior pattern. The forager ant followed the flow of traffic. Small ants (most likely gardeners/nurses based on size) moved non-specifically near the entrances. They moved between different leaves, touching them and then moving to the next leaf. When a leaf was rejected, instead of moving away, a small ant would approach the forager ant and touch it. The forager ant moved to the size of the path, and other small ants continued to approach it, and would either stay with it or move away. Once a certain number of small ants congregated around the leaf, the forager would lay it down and all the ants would move away. After the leaf was dropped, usually another group of small ants approached with 30 seconds to drag it a few centimeters farther from the path. This behavior pattern was followed in seven of the rejection events. In the other rejection event, only a few small ants

stayer with the forager ant. Other small ants occasionally came to touch the leaf fragment, and the forager ant slowly lowered the fragment.

DISCUSSION

The results of this study suggest that leaf size is not a considerable selection filter near the *A. cephalotes* colony. However, the number and total mass of leaves decreased with distance from the colony, which supports the hypothesis that there is a selection filter near the colony that differs from selection closer to the harvest site. There may be other selection factors that are more dominant near the colony that this study was unable to address. For example, North et al. (1997) found that leafcutters ants can modify which types of leaves they bring into the colony based on the current needs of the fungi. It is possible that instead of size, the selection filter near the colony is based on leaf composition, to meet the needs of the fungi. If this is the case, the types of leaves dropped near the colony may change over time and differ substantially between colonies, even within the same species. However, since there are so many different species of plants in the neotropics, it may be difficult to classify leaves harvested by the ants to the species level. Future studies could instead investigate the chemical composition and microbial community of rejected leaves.

Disturbances may also cause leafcutter ants to abandon their leaves, which could have confounded the results of the leaf fragment analysis. *Atta colombica* has been shown to drop leaves more frequently when it is raining (Hodgson 1955). Since it rained during two of the three days that I collected leaf fragments, some of the leaf fragments I collected on those days may have been dropped due to disturbance rather than rejection. Additionally, the leafcutter colony I investigated was next to a road with frequent foot and vehicle traffic, and the major leafcutter

path crosses the road. Human activities, particularly when the leafcutter colony is most active at night, may also cause the ants to drop their leaf fragments.

My observations of leafcutter ants at CCI and La Cotinga in Puntarenas Providence, Costa Rica, suggest leafcutter ants are generally resistant to disturbance, and will usually pick their leaf fragments back up if a disturbance causes them to drop the fragment. In the videos of leafcutter ant behavior at CCI, I noted numerous examples of ants dropping their leaf fragments momentarily but picking them back up, except in the eight events I classified as rejection. Additionally, at La Cotinga I observed leafcutter ants (*A. colombica*) harvesting leaf fragments from a tree. Most of the ants were in the tree, but there were some interspersed along the ground. Occasionally, an ant in the tree dropped a leaf fragment. The ants on the ground seemed to be searching for and collecting drops leaf fragments, and carrying them back to the ant path. These observations suggest that ants will exert considerable effort to recover lost leaf fragments unless the fragments are intentionally dropped.

My observations suggest that at least some instances of leaf fragment loss are driven by rejection rather than disruption. My observations support the hypothesis that there is a selection filter near the colony that selects for different leaf qualities than filters closer to the harvest site. Since the leaf fragments were only rejected after contact with several other ants, they seem to select leaves through a form of quorum voting. Additionally, the ants that made contact with the fragments and helped to move them out of the ant path were morphologically distinct from the ants carrying the leaf fragments. The differences in size between these types of ants suggests that a separate caste of ants is responsible for implementing this selection filter by checking each leaf fragment before it enters the colony. The size of the smaller ants is consistent with those described by Wilson (1980) as gardeners/nurses. The morphological similarity suggests that

these small ants part of the gardener caste and perform an additional role in leaf fragment selection.

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