Leafcutter Ants Inside The Nest Have Sharper Mandibles Than Ants Outside The Nest

David M. Lee
Biochemistry, Biology

Mentor: Robert Schofield
Physics

Introduction

Leafcutter ants are both dominant consumers and valuable contributors to ecosystems throughout the neotropics.[1-3] They harvest small disks of leaves (and other materials) from their environment, cut them into smaller fragments, and use the fragments as substrate for the gardens of fungi they grow as a food source.[4-7] Therefore, leafcutters as producers of a crop are farmers. The amount of energy provided by the crop limits the total net growth of the colony, much like the growth of a factory is limited by the amount of money brought in from production. The colony must be aware of how much energy it uses[8-11] versus the amount of energy the fungal gardens provide.[10,11] With positive net energy the colony grows, but with negative net energy growth stops. If the energy budget goes too far into the red, the colony begins to collapse. Leafcutter ants

have made their living in environments of bustling tree canopies and grasslands. Even with the heavy labor of harvesting materials, processing the substrate, and tending meticulously to their fungal gardens, leafcutter ants and their mycological symbiont have conquered most of Latin America.[4-6]

Of all the cutting that leafcutters do, 90% occurs inside of the nest.[11] Leafcutters use a pair of toothed mandibles to do their cutting and rely on two techniques: leading-lagging for cutting soft materials and saw-action for cutting tough materials.[10,11] As a leafcutter ages, its mandibles wear down without regeneration. Consequently, an ant with worn mandibles will spend twice the energy and will cut half as fast as an ant with sharp mandibles.[10] Smaller organisms, such as leafcutter ants, are able to prolong the sharpness and efficiency of their biotools in part because those critical body parts are enriched by heavy metal and halogen (Zn, Mn, Br).[13] Moreover, the allocation of tasks based on wear may also play a significant role in prolonging the optimal cutting efficiency of a leafcutter's mandibles while contributing to the viability of the colony.

Methods

We suspected that ants inside the nest, where most of the cutting happens, would have the sharpest mandibles. To investigate this

hypothesis, we analyzed four groups of media *Atta cephalotes* collected from a two-year-old colony in Minca, Colombia (2012): one group from inside the nest and three groups from outside the nest. Our study compared the tooth lengths of each ant to the lengths of a similar-size late-stage pupa. Late-stage pupae are ideal for comparing tooth wear because they have pristine, fully developed mandibles. By subtracting the tooth lengths measured in the lab from the tooth lengths of a late-stage pupa and averaging the wear values of all measured teeth per ant, we calculated the average amount of tooth that had worn over the ant's life.[10] We then compared the results from individual groups to one another.

Specimens

Groups consisted of *Atta cephalotes* collected from fungal gardens inside the nest (Inside Ants, $n = 79$) and three groups from outside the nest: 1) ants cutting leaves (Leaf Cutters, $n = 81$), 2) ants cutting a mango (Mango Cutters, $n = 43$), and 3) ants carrying leaves (Carriers, $n = 79$). Our selection criteria called for only media ants to be collected. Groups were stored in a glass vial with ethanol as a storage medium. Using similar-sized late-stage pupae as comparators, tooth lengths were calculated using the methods described in Schofield et al.[10]

Dissection and Photography Protocol

Each head and mandible was photographed using a Fujifilm FinePix S2950 digital camera shot through a Zeiss dissection microscope. The head and mandibles were photographed separately with their superior surfaces parallel to the plane of the stage.

Head Width Photo (Figure 1). Each ant was decapitated, and the antennae and mouthparts were removed with tweezers. The head was oriented in the middle of the optics, and a millimeter scale was placed behind it. A "head-width" photo was captured. The closure apodeme for each mandible was then severed using a No. 11 scalpel. Severing the apodeme was necessary to allow the mandible to be manipulated across its full range of motion.

Mandible Photo (Figure 1). The head was placed in a groove on a clay pedestal, and the left mandible was positioned using a toothpick. Proper orientation required that the superior surface of the mandible be parallel to the plane of the stage. To assure that the mandible was positioned correctly, strobing light from an LED flashlight was shone through the eyepiece to allow a visual confirmation that the mandible was level. Strobing light glinting evenly across the surface of the mandible acted as a visual indicator that the sample was near-parallel to the plane of the stage. With the sample

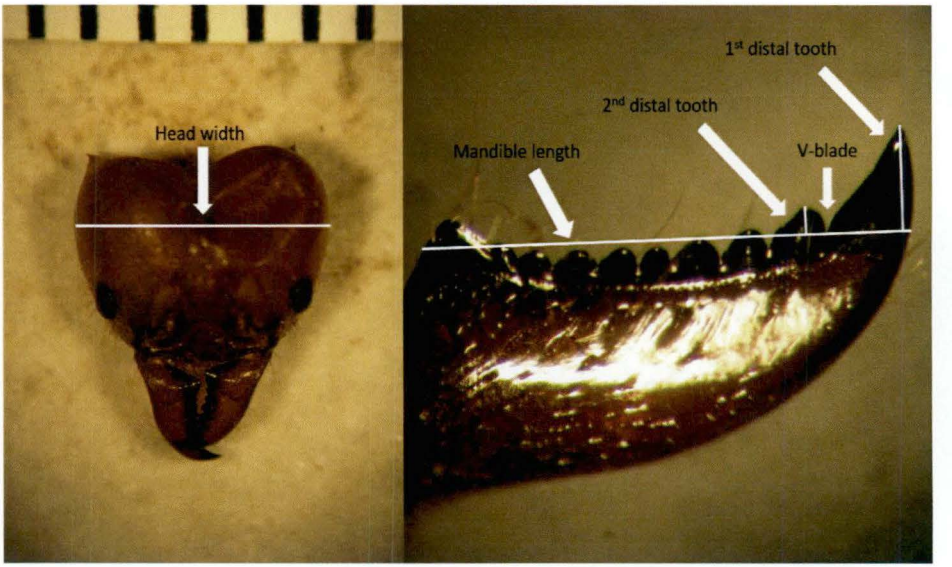


Figure 1. Measuring head width (left) and mandible (right) photos. Solid lines represent measurements taken on ImageJ. Dashed lines represent scalpel cuts.

oriented correctly, a "left mandible photo" was captured using the optical zoom of the camera at 1.8X and the microscope zoom at 5X. A photograph of a millimeter scale was taken at this zoom for data analysis. The mandible photo process was repeated for the right mandible.

Photographic Analysis

Photographic measurements were recorded using ImageJ photo analysis software version 1.50i. A head width measurement was considered the length of the longest line that could be drawn across the top of the head while remaining parallel to a line connecting the

center of the eyes (Figure 1.a). A mandible length measurement was considered the length of a line that connected the anterior edge of the first distal tooth to the proximal edge of the last tooth by passing through the bottoms of the valley created by the V-blade and the valley formed at the interface of the last two teeth. Distal tooth measurements were considered the length of a line connecting the tip of the tooth perpendicularly to the mandible length line (Figure 1.b).

Data Analysis

First and second distal tooth measurements were compared to a similar-size late-stage pupa using Equations 1 and 2, respectively. The difference between the tooth lengths from our measured sample (T_1 & T_2) and those calculated for a late-stage pupa (bracketed portion of Equations 1 & 2) quantified the amount of wear per tooth (W_1 & W_2). Wear computed from the first distal tooth of the left and right mandible was regressed to the status of a second distal tooth with proportional levels of wear to account for biases in wear rates. The regressed teeth were averaged in Equation 3 with the measured levels of wear from the second distal teeth to compute the average wear ($\overline{W}$). Groups were binned per average mandible length, .225 mm to .900+ mm in .075 mm increments. Bins were normalized using an online random number generator (https://www.random.org) to elim-

Weighted Average Wear Computation

L_M = mandible length, T_1 and T_2 = measured tooth lengths, W_1 and W_2 = calculated tooth wear, $\overline{W}$ = weighted average wear

1. $[.2648L_M - .0308] - T_1 = W_1 \quad \text{for} \quad W_{1L} \,\&\, W_{1R}$

2. $[.0533L_M + .0301] - T_2 = W_2 \quad \text{for} \quad W_{2L} \,\&\, W_{2R}$

3. $$\frac{\frac{W_{1L} + W_{1R}}{2.61} + W_{2L} + W_{2R}}{4} = \overline{W}$$

Figure 2. Wear equations from Schofield et al.[10]
Equation 1. Difference in length, late-stage pupa minus measured: 1st tooth.
Equation 2. Difference in length, late-stage pupa minus measured: 2nd tooth.
Equation 3. Average Wear: regressed 1st teeth and 2nd teeth.

inate individual data points. Wilcoxon-Mann-Whitney Test results were computed using an online calculator (www.ccb.uni-saarland.de/wtest).

ESEM Protocol

Electron micrographs were captured on an FEI Quanta 200 ESEM/VPSEM in low vacuum mode ($P \leq 20$ Pa) with low voltage ($V \leq 10$ kV) over a 90 second scanning interval. The bottom 10% least worn mandibles from individuals of each group were selected to compare minute levels of wear at higher magnification. The mandibles

were dissected completely from the head and mounted parallel to each other on a circular mounting pin with the plane of the first distal tooth's proximal edge oriented normal to the stage. Uncoated samples had been air dried prior to scanning, and the mount had been prepared with an adhesive carbon disk to aid in stabilizing the sample and decreasing charge levels. Electron micrograph measurements were recorded using ImageJ photo analysis software version 1.50e.

Results

Preliminary findings revealed that 95% of ants outside the nest had wear that was more than .025 mm, and 70% of ants inside the nest had wear that was less than .025 mm (Figure 3). Ants found inside the nest had mandibles that were sharper than those of ants found outside the nest by a factor of 2.14 (p = 5E-18) (Table 1).

Wear Comparison	n per group	Test	p-value	$\frac{\overline{W}_{out}}{\overline{W}_{in}}$
Inside Ants vs. Outside Ants	72	H_1 = in < out	4.67^{-18}	2.14
Inside Ants vs. Carriers	34	H_1 = in < out	1.70^{-16}	2.42
Inside Ants vs. Leaf Cutters	34	H_1 = in < out	1.80^{-10}	2.32
Inside Ants vs. Mango Cutters	34	H_1 = in < out	9.60^{-08}	1.98

Table 1. Results from Wilcoxon-Mann-Whitney Test are shown with exact p-values and group average wear ($\overline{W}$) ratios (outside over inside).

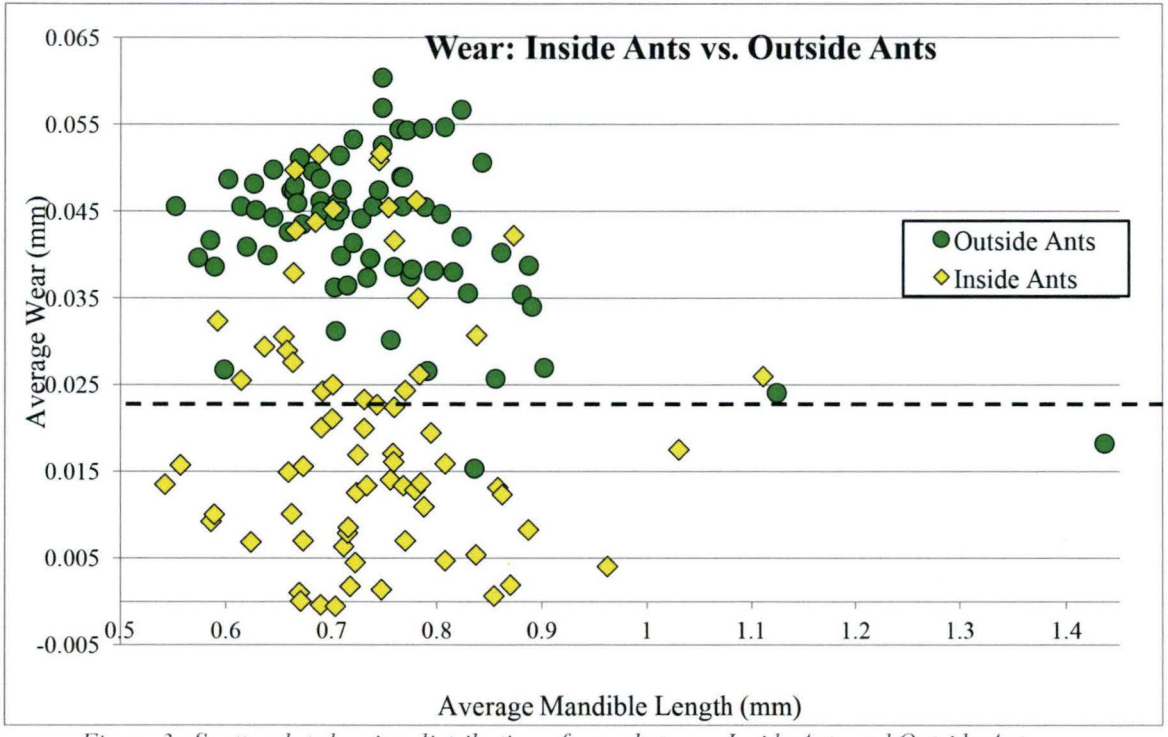


Figure 3. Scatterplot showing distribution of wear between Inside Ants and Outside Ants. Dashed line = .025 mm average wear. n = 72 per group.

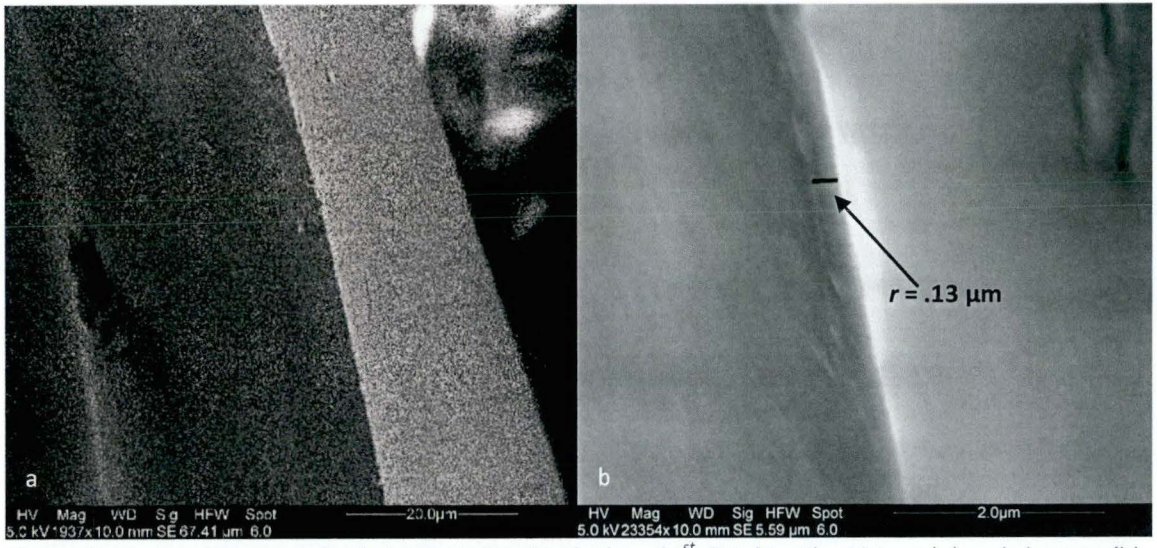


Figure 4. Electron micrographs: Inside Ant. Proximal edge of 1st distal tooth. Distant (a) and close-up (b) zooms. Black line represents diameter measurement taken on ImageJ.

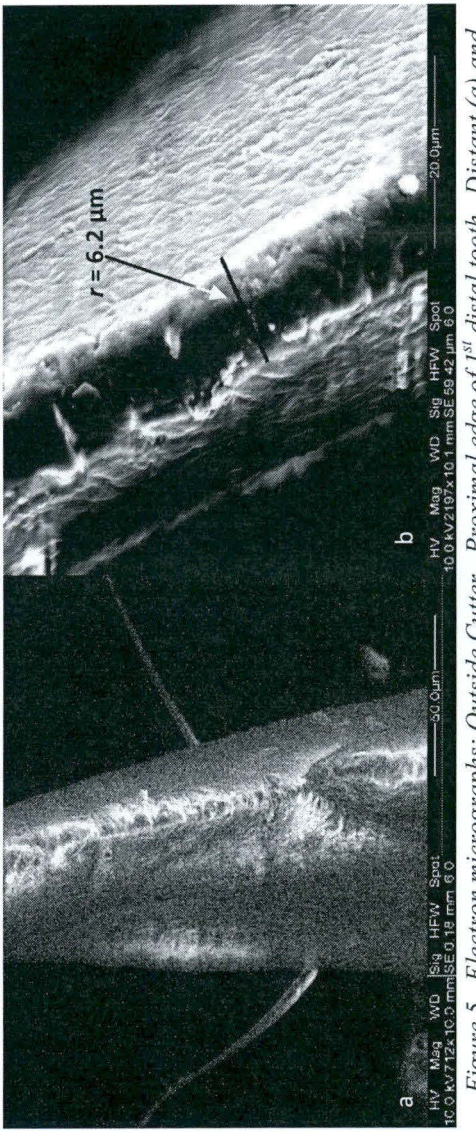


Figure 5. Electron micrographs: Outside Cutter. Proximal edge of 1st distal tooth. Distant (a) and close-up (b) zooms. Black line represents diameter measurement taken on ImageJ.

Discussion

Preliminary findings reveal that leafcutter ants inside the nest have sharper mandibles than ants outside the nest. The result we obtained was simple but carries large implications. It costs a lot of energy to make and maintain an ant.[8-11] The colony would risk losing that investment of energy by having the ant immediately leave the safety of the nest after ecdysis to work outside, but the colony still needs to be continually sending workers out to harvest materials for substrate to feed to their fungal gardens. Leafcutters may spend the first portion of their lives working inside the nest when they are the most efficient at cutting. Once their mandibles wear to a certain point, it will take an ant twice the energy to cut the same amount of material.[10] This decreased efficiency may be enough for the colony to risk losing the ant to the dangers of the outdoors.

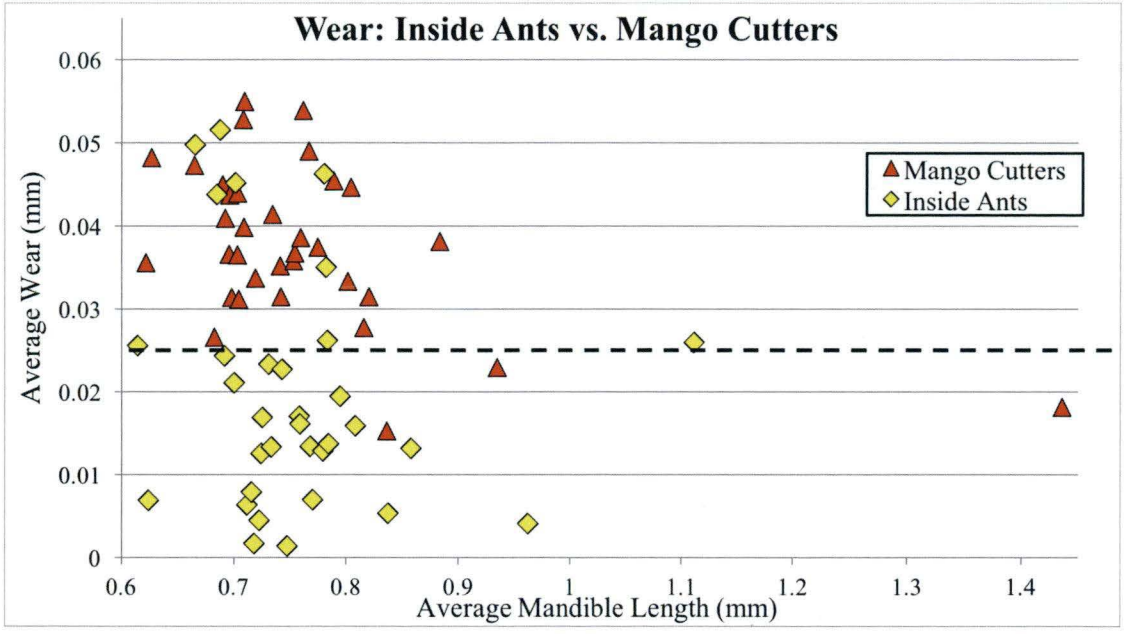
The electron micrographs in Figure 4 and Figure 5 show the difference in wear between an ant selected from inside the nest and an ant selected from outside the nest. The sharpness of a blade is dependent on the radius of the blade's tip. A smaller radius will contact less material, constituting a sharper blade.[12] In this comparison, both ants possess mandibles that are in the bottom 10% (least worn) from their respective groups although the inside ant has a cutting

blade that is 50 times sharper than the cutting blade of the outside ant. This difference in sharpness is comparable to cutting a steak using a sharp steak knife versus a dull butter knife. The sharper knife requires less energy and allows the task to be completed in less time. Keeping the sharpest mandibles inside the nest may be one way the colony chooses which ants work inside the safety of the nest and which ants must risk their lives by foraging outside of the nest. Our data suggest that an ant starts to work outside the nest after .025 mm of its mandibles have worn away. However, we are uncertain whether this observed behavior is in response to wear or in response to the longevity an ant has left to give to the colony. One could test this by seeing if young ants with especially worn mandibles leave the nest to forage.

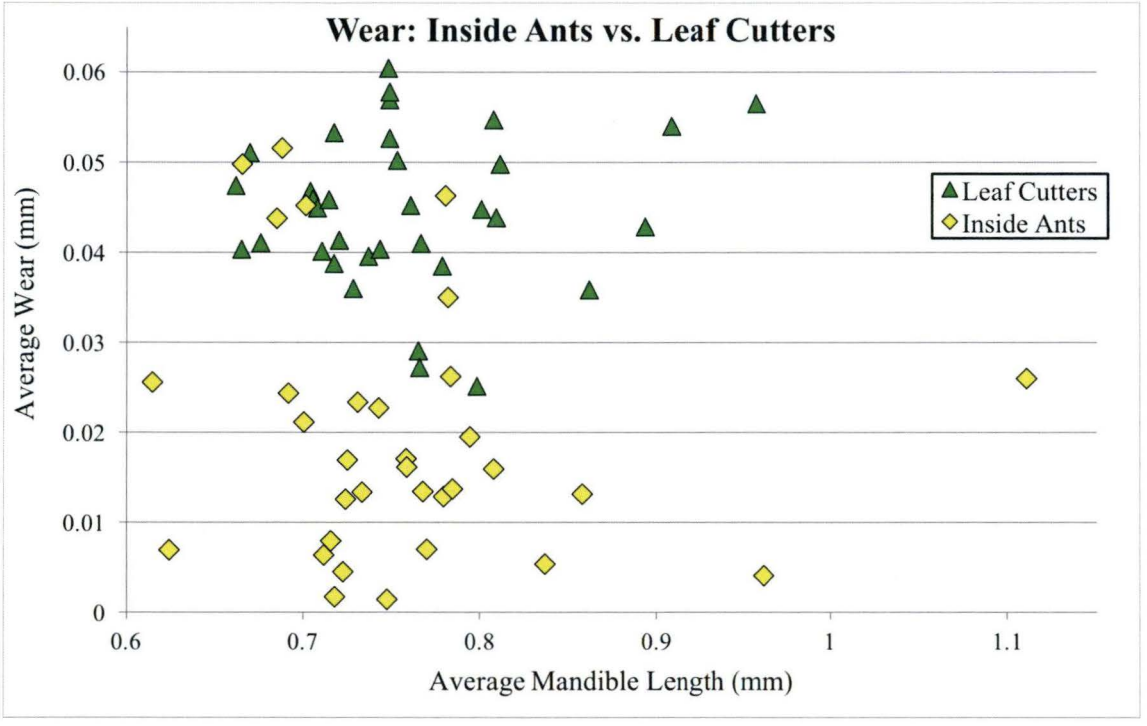
Acknowledgements

I would like to thank Dr. Robert Schofield for birthing the project and guiding me toward its completion; Kate Carlson, Ryan Garrett, and Matthew Goggans for their intellectual contributions; my fellow McNair scholars for taking this ride with me; and Dr. Gail Unruh for always going above and beyond.

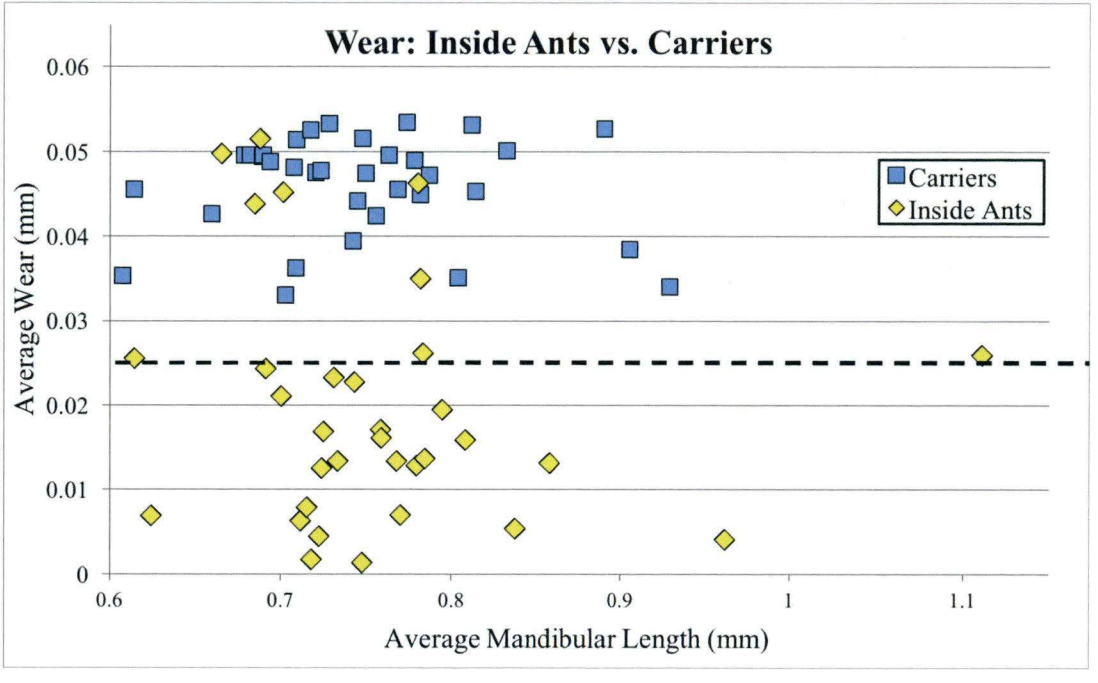
Supplemental Materials



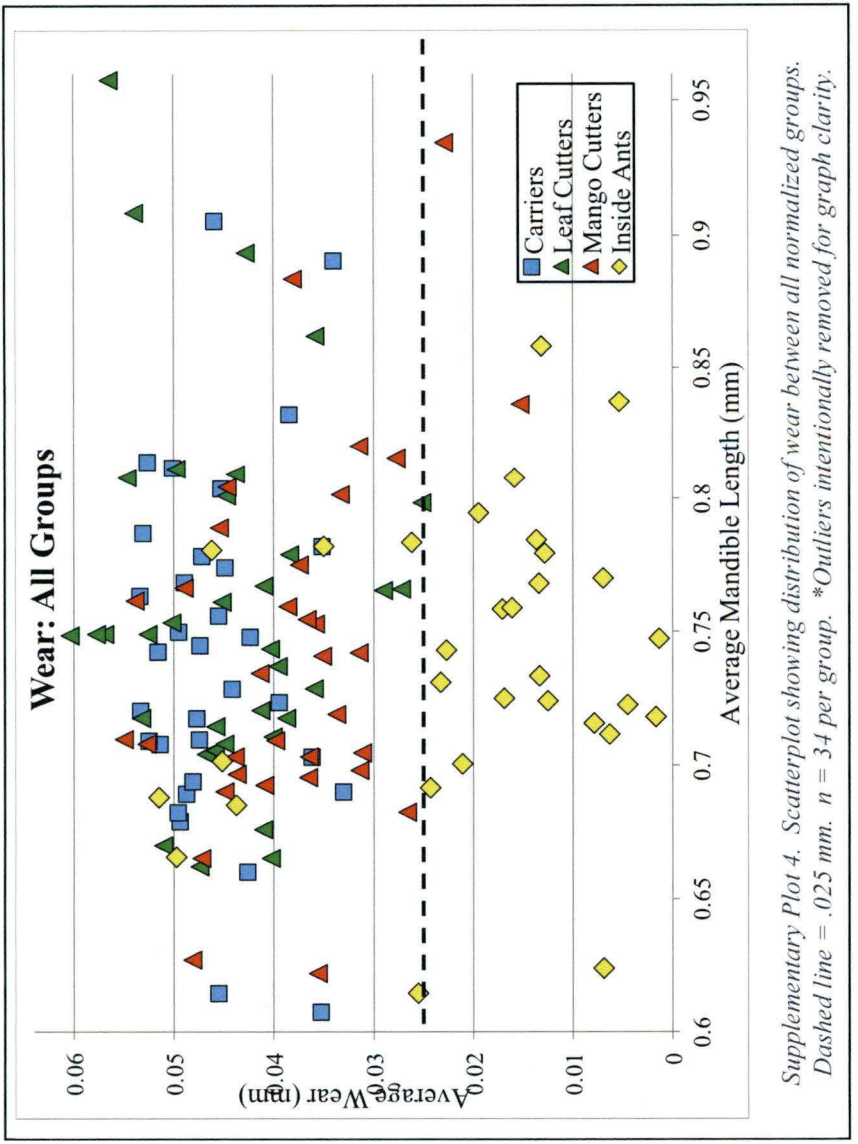
Supplementary Plot 1. Scatterplot showing distribution of wear between Inside Ants and Mango Cutters. Dashed line = .025 mm. n = 34 per group.



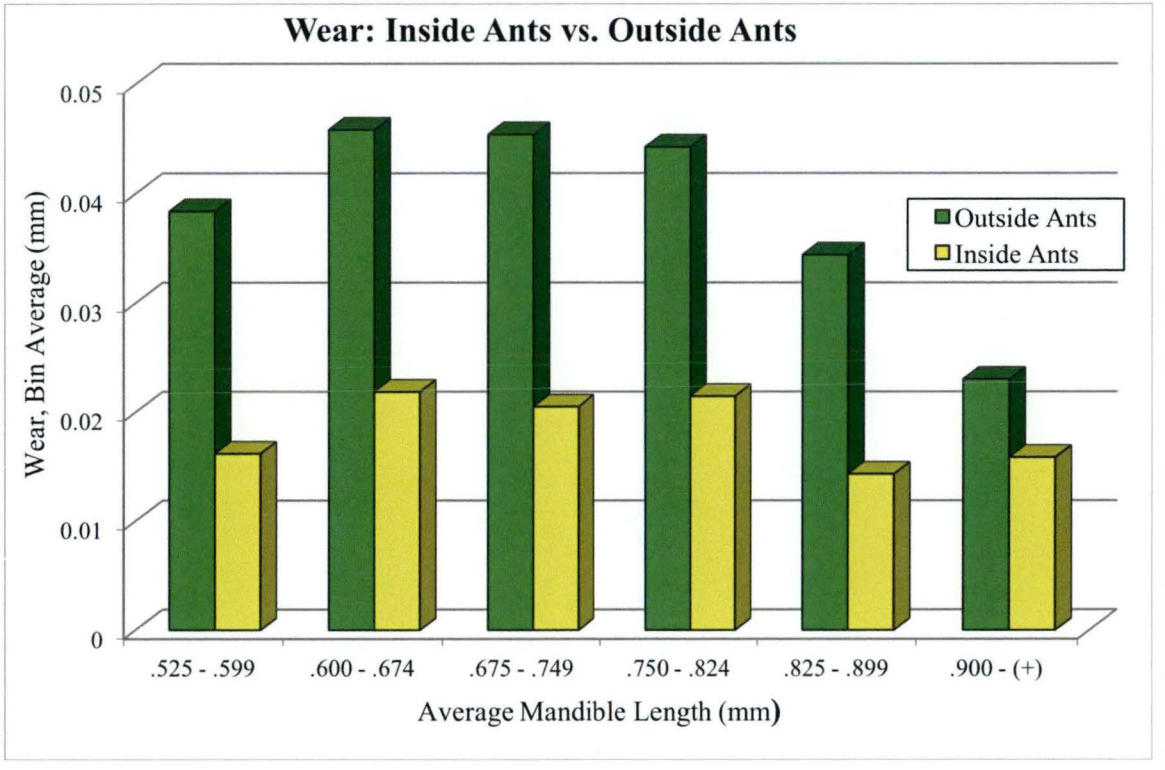
Supplementary Plot 2. Scatterplot showing distribution of wear between Inside Ants and Leaf Cutters. Dashed line = .025 mm. n = 34 per group.



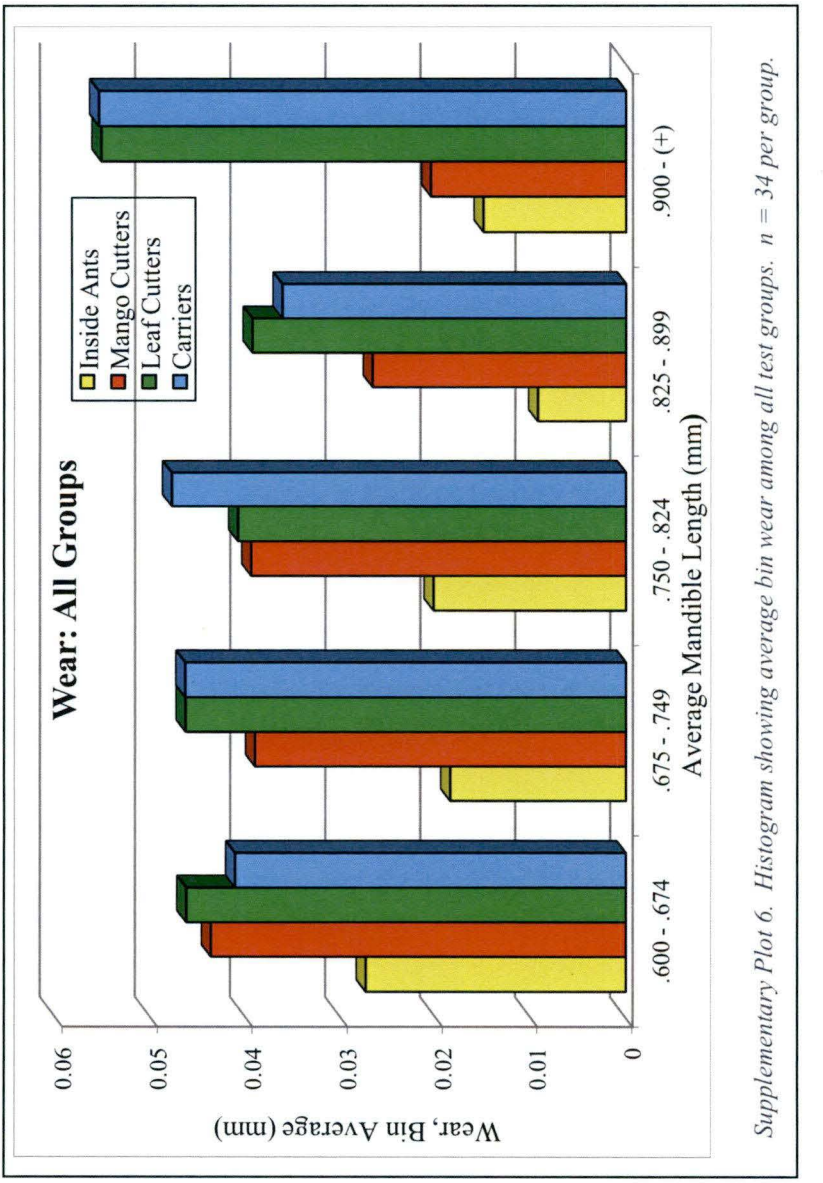
Supplementary Plot 3. Scatterplot showing distribution of wear between Inside Ants and Carriers. Dashed = .025 mm. n = 34 per group.



*Supplementary Plot 4. Scatterplot showing distribution of wear between all normalized groups. Dashed line = .025 mm. n = 34 per group. *Outliers intentionally removed for graph clarity.*



Supplementary Plot 5. Histogram showing average bin wear between Inside Ants and Outside Ants. n = 72 per group



Supplementary Plot 6. Histogram showing average bin wear among all test groups. n = 34 per group.

Wear Comparison (a vs. b)	*n* per group	Test	*p*–value	$\frac{\overline{W}_b}{\overline{W}_a}$
Inside Ants vs. Outside Ants	72	$H_1 = a < b$	4.67^{-18}	2.14
Inside Ants vs. Carriers	34	$H_1 = a < b$	1.70^{-16}	2.42
Inside Ants vs. Outside Cutters	62	$H_1 = a < b$	2.31^{-14}	2.07
Inside Ants vs. Leaf Cutters	34	$H_1 = a < b$	1.80^{-10}	2.32
Inside Ants vs. Mango Cutters	34	$H_1 = a < b$	9.60^{-08}	1.98
Mango Cutters vs. Carriers	34	$H_1 = a \neq b$	5.25^{-05}	1.22
Mango Cutters vs. Leaf Cutters	34	$H_1 = a \neq b$	0.0119	1.17
Outside Cutters vs. Carriers	62	$H_1 = a \neq b$	0.0346	1.07
Leaf Cutters vs. Carriers	34	$H_1 = a \neq b$	0.699	1.05

Supplementary Table 1. Results from Wilcoxon-Mann-Whitney Test are shown with exact p-values ($p > .05$ denoted in red) and group average wear ($\overline{W}$) ratios (b over a) for an array of comparisons

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