Pheromones and Path Selection of *Atta cephalotes*

Joshua Coon
Biology

Mentor: Robert Schofield
Physics

Introduction

Researchers R. Beckers, J. Deneubourg, and S. Gross outlined the current methodology focusing on path choice when they wrote: "If a mass forager arrives at a fork in a chemical recruitment trail, the probability that it takes the left branch is all the greater as there is more trail pheromones on it than the right one, adding more pheromones increasing the probability of the next forager to choose the left trail"[12]. This methodology is troubling because it does not account for the energetics behind the day-to-day tasks of ants.

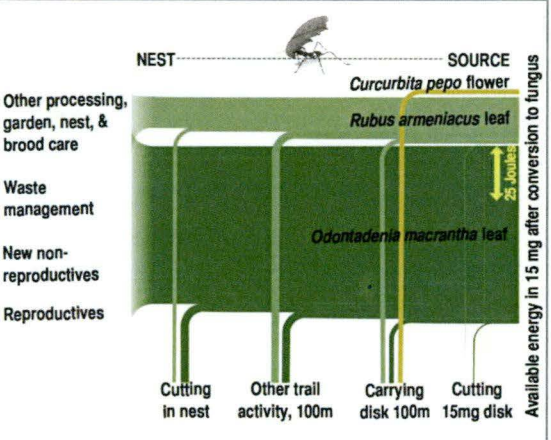

Figure 1. A preliminary energy budget for foraging, demonstrating large differences in energy profits for different tissues (colors). Shown is the available energy before (right side) and after (left side) cutting, transport, and further cutting in the nest. Because of high water content, many flowers contain less energy than required to transport them to the nest. Other costs of foraging per 15 mg load (other trail activity) are estimated from the sampled ratio of carriers to non-carriers on a foraging trail and the energy usage of non-carriers. The preliminary estimates at the bottom were made using bomb calorimetry and respirometery.

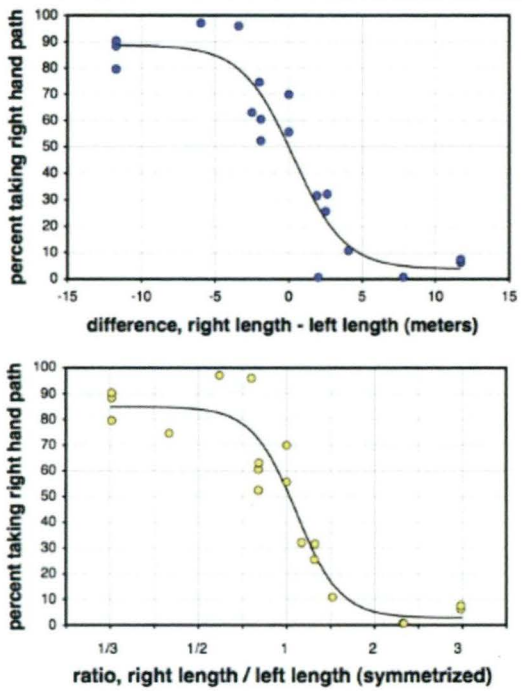

Figure 1. Data (points) and fits (lines) for 18 preliminary path selection experiments. 75% or more of the transporters took the shorter path if the difference was 2.5 m or more, and the ratio was 1.4 or greater.

Work in the Schofield lab at the University of Oregon (figures 1, 2) shows that ants can indeed choose a different path than the one with the largest quantity of pheromones and also have a multitude of tasks to accomplish for a successful colony to be productive and healthy. Figure 1 shows the usages of energy provided by three types of sources. The graph displays the amount of energy (joules/thickness of leaf section taken) required for each listed task. Figure 2 shows the path selection of ants during a timed trial with two sections of tube. As part of the trial, one section of tube was replaced by a section of shorter, clean tubing. As observed, the ants chose the path that was at least 2.5m shorter. In light of the newer work focused on energetics, the trials in the Schofield lab reveal that the ants do indeed choose a path that is shorter, or, in other words, the ants choose the more energy efficient path. According to the current and more traditional methodology, ants would be unable to switch to a shorter path, and the observations we have seen would be impossible. Based on observations in the lab, I propose that, instead of mass pheromonal control,

there is selective pheromonal control over the direction and path that the ants take and that choice depends on whether the ants are foraging (non-leaf carrying) or leaf carrying[8].

Methods

Designing and building a structure that allows for the selection of an individual classification of ant (leaf carrying or non-leaf carrying) with simplicity[5,10,9] is challenging because of the inherent nature of ants, which includes their always attempting to explore the surrounding area. The structures were mostly made of polycarbonate and other thick mendable plastics. Pieces were mended together using Methyl Ethyl Ketone (MEK), a common solvent. Three main pieces composed the track: separation chamber (A), collection chamber (B), and one-way chamber (C). A talc powder coated the sides of the chambers to restrict the ant flow to a linear motion and not allow them to roam off of the path. Along with the control

Figure 3A - Separation chamber allowing selection by category of ant.

Figure 3B: Teflon bridge and holding structure to maximize ants on bridge.

Figure 3C: One-way chamber control the direction of travel.

of the talc powder, double-sided tape was placed at the four legs of the table and along the secondary containment chamber to insure that the ants were unable to completely escape even if a breakout occurred.

Collection

The selection and collection of the pheromones followed two scenarios: ants carrying leaves and ants not carrying leaves. To accomplish this selection, the separation chamber was set at a height that allowed the use of insect forceps to pick out ants that were carrying leaves, thus selecting for ants that were not carrying leaves. The procedure was reversed to select for ants carrying leaves so that ants not carrying leaves were removed. A piece of plastic in the separation chamber converted the chamber into a very tight hallway that did not allow the ants carrying leaves to pass through. Once the target ants were selected, they walked through to the collection chamber that houses the Teflon half pipe. The ants walked across the half pipe for two hours depositing their pheromones. Teflon, because of its chemical properties that make it resistant to chemical attack, is easy to rinse with a solvent to clean the deposited chemical. The washing of the half pipe was conducted using HPLC grade hexanes, setting the half pipe to a steep angle and running approximately 1.5ml of the hexane down the half pipe twice. Once the wash had been completed, the collected solvent was extracted from the container using a 1ml syringe and filtered into a GC-vile using a 0.2μm PTFE filter. One important feature of the experiment are the two different Teflon half pipes that were used during the trials. The whole apparatus was set up 24 hours prior to the collection using an "acclamation" half pipe; letting this time pass ensured a good quantity of traffic over the bridge. Once the run was initiated, the acclamation half pipe was switched with the "collection" half pipe. Once the pipes were switched, the selection process began, and the ants ran along the Teflon bridge for a minimum of two hours.

Characterization

Once the solvent containing pheromones had been filtered properly, it was run on a GC-MS[1,4,9,6] with the assistance of Daniel Seidenkranz. The following section is the method of treatment for each vial containing the solvent and analyte.

Pre-run Program	Initial Settings	GC Program	MS Table
Number of Rinses with Pre-solvent: 2	Column Oven Temp.: 70.0 °C	Ion Source Temp: 200.00 °C	Start Time: 3.00min
Number of Rinses with Solvent (post): 2	Injection Temp.: 275.0°C	Interface Temp.: 275.00 °C	End Time: 22.23min
Number of Rinses with Sample: 2	Injection Mode: Split	Solvent Cut Time :2.50 min	ACQ Mode: Scan Event Time: 0.30sec
Plunger Speed (Suction): High	Flow Control Mode: Pressure	Detector Gain Mode: Relative to the Tuning Result	Scan Speed: 1666
Viscosity Comp. Time: 0.2 sec	Pressure: 100.0 kPa	Detector Gain: 0.88 kV +0.00 kV	Start m/z: 35.00
Plunger Speed(Injection): High	Total Flow: 52.0 mL/min	Threshold: 0	End m/z: 500.00
Syringe Insertion Speed: High	Column Flow: 1.53 mL/min		
Injection Mode: Normal	Linear Velocity: 45.4 cm/sec		
Pumping Times: 5, Inj.	Purge Flow: 3.0 mL/min		
Port Dwell Time: 0.3 sec	Split Ratio: -1.0		
Terminal Air Gap: No			
Plunger Washing Speed: High			
Washing Volume: 8uL			

Total time: 22.33 min.

Results

For control using the GCMS, a sample of pure hexanes HPLC was run through clean Teflon halfpipe and filtered through a Teflon filter in order to get a baseline of the peaks that may occur from backline pollution. Graphs A-J (Figure 4) were obtained using the methods outlined above. The re-

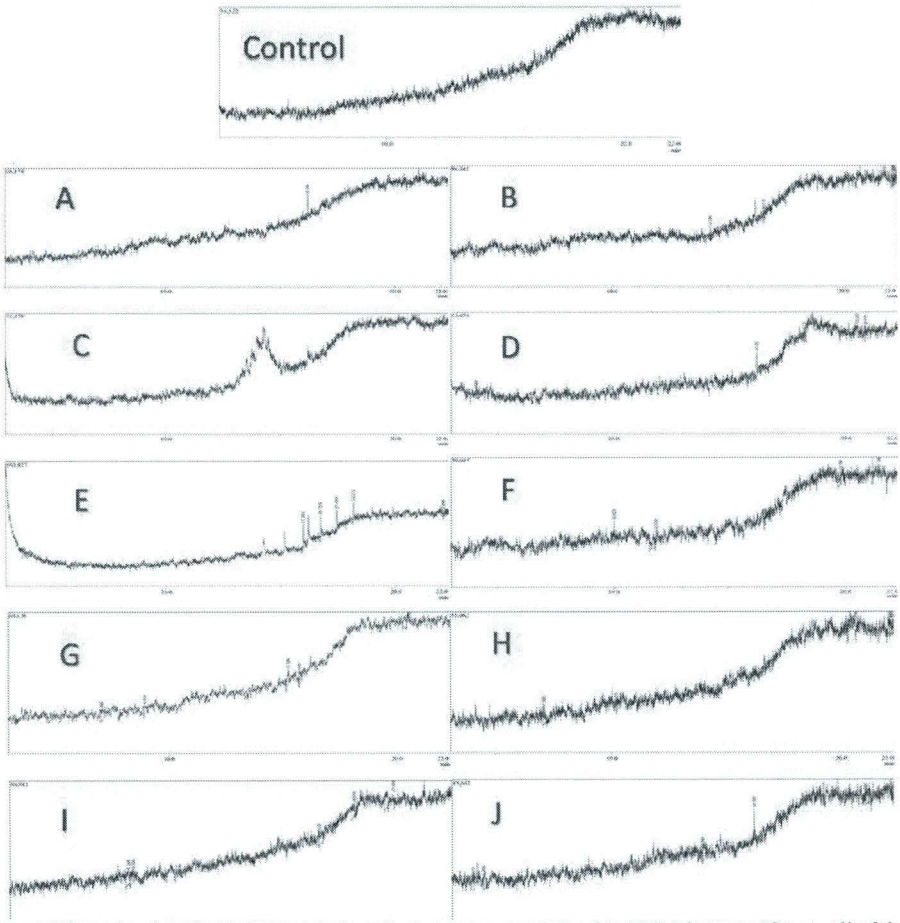

Figure 4 Control, A-J: Preliminary date for samples run on GC-MS using HPLC hexane solvent. All of the samples are a combination of leaf carrying and non-leaf carrying ants in order to maximize pheromone concentration. All graphs showing no significant concurrent peaks, however a potential peak at 16min may be observed after further optimization. Figure-C shows contamination from the previous run done on GC-MS unrelated to pheromones.

sults shown in figure 4 reveal that there are potential peaks that coincide with the sample analyzed in the GC-MS column, but no peaks that appear repeatedly.

Discussion

Lacking reproducible peaks during the analysis of the samples means that one of two changes must be made. Switching to a split-less GC-MS method would allow for a much more sensitive characterization of the analytes that are in the prepared sample. The other new procedure that could compensate for the lack of sensitivity available on the current GC-MS machine would be to optimize the track to its fullest. Measures are underway to improve the amount of pheromones that are deposited and the purity of pheromones that can be extracted. Along with the Teflon tubing, glass fibers and filter paper will be used as a path for the ants to walk along and deposit pheromones. The idea behind the use of the fibers is that the Teflon is potentially resistant to chemical alteration, and it is possible that the secreted pheromones remain on the abdomen of the ant rather than being deposited onto the track. The glass fiber and filter paper will act as a type of sponge, allowing for the pheromones to wick off of the abdomen and be held by the material. Another optimization property that the glass fiber and filter paper enable is the possibility that purity of the collected sample would be greater. Instead of allowing the substrate to come into contact with the environment, it is now taken straight from the track and placed into a vial containing the HPLC hexanes and sealed. The sample can then be drawn directly from the vial and put into the GC-MS vial. Another method for optimization is the use of the chemical differences between the potential pheromones and the HPLC hexanes. The hexanes are small molecules with high evaporation levels at standard room temperature, but trail pheromones are larger molecules that do not vaporize for approximately

24 hours[3,11]. Because of evaporation, there will be a greater likelihood of a maximized amount of pheromones with the hexanes that would, in turn, allow for a greater probability for the pheromones to be picked up by the detector. Once the optimization is great enough and reproducible peaks are obtainable, this research may change the current methodology on path choice and the selection process the ants go through in picking the paths.

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