

The Effect of Lipids on Attine Ant Behavior

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Introduction

Lipid metabolism is an integral biological system of life. Within a variety of taxa encompassing multiple kingdoms, lipids are shown to be vital for an array of functions. Specifically, this project aims to create a more detailed understanding of the function of lipids as allelochemicals in symbiotic relationships. This projects uses attine ants and their host fungus as a model for symbiosis and lipid analysis.

Three specific lipids have been identified in different regions of the attine biological system. Specifically, α-linolenic acid, linoleic acid, and oleic acid have been found in the fresh plant matter, the fungus gongylidia, and perished ants respectivly⁵. This project has compared the behavioral reactions of A. texana ants to these three different lipids, allowing for an analysis of how lipids may be used as a potential allelochemical in this species.

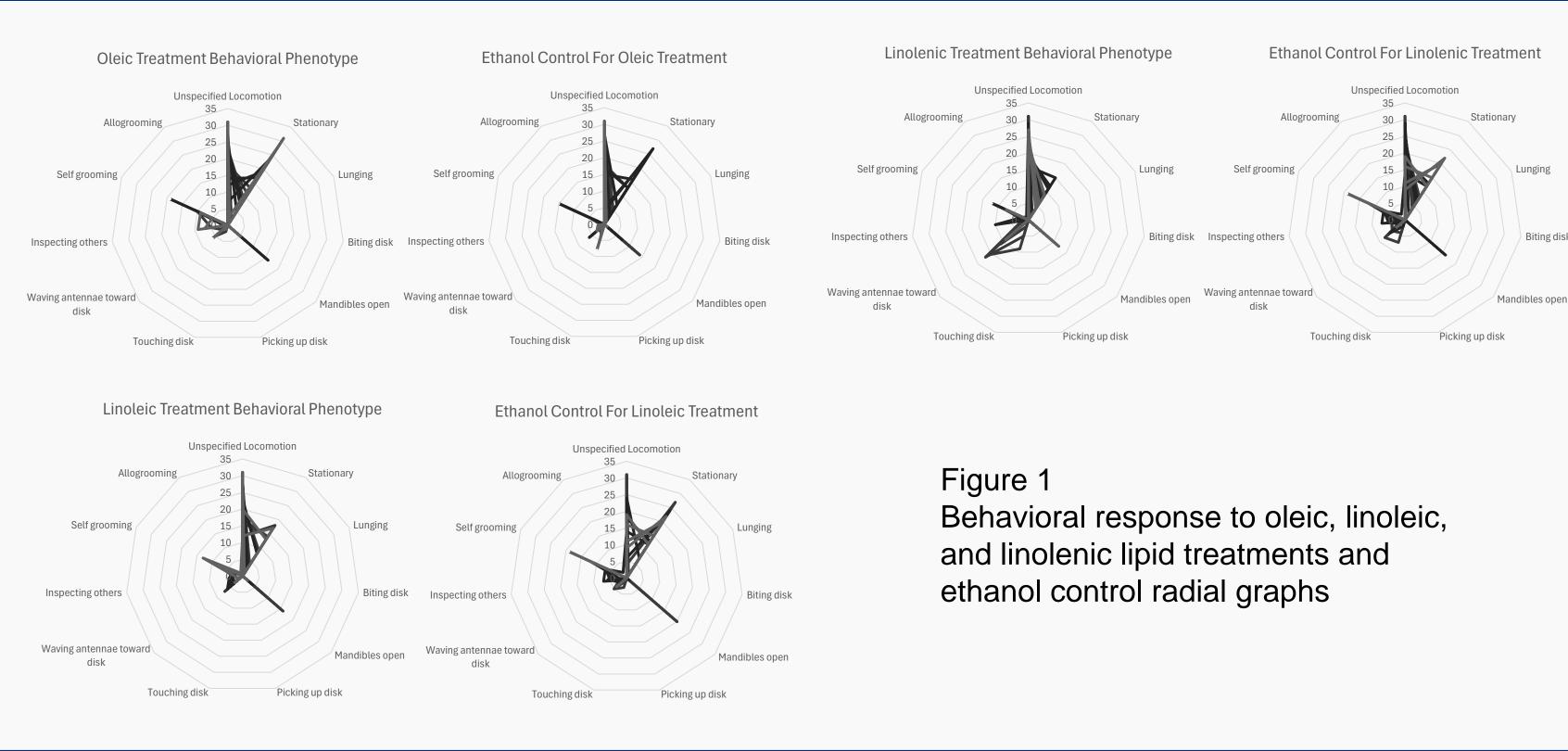
Methods

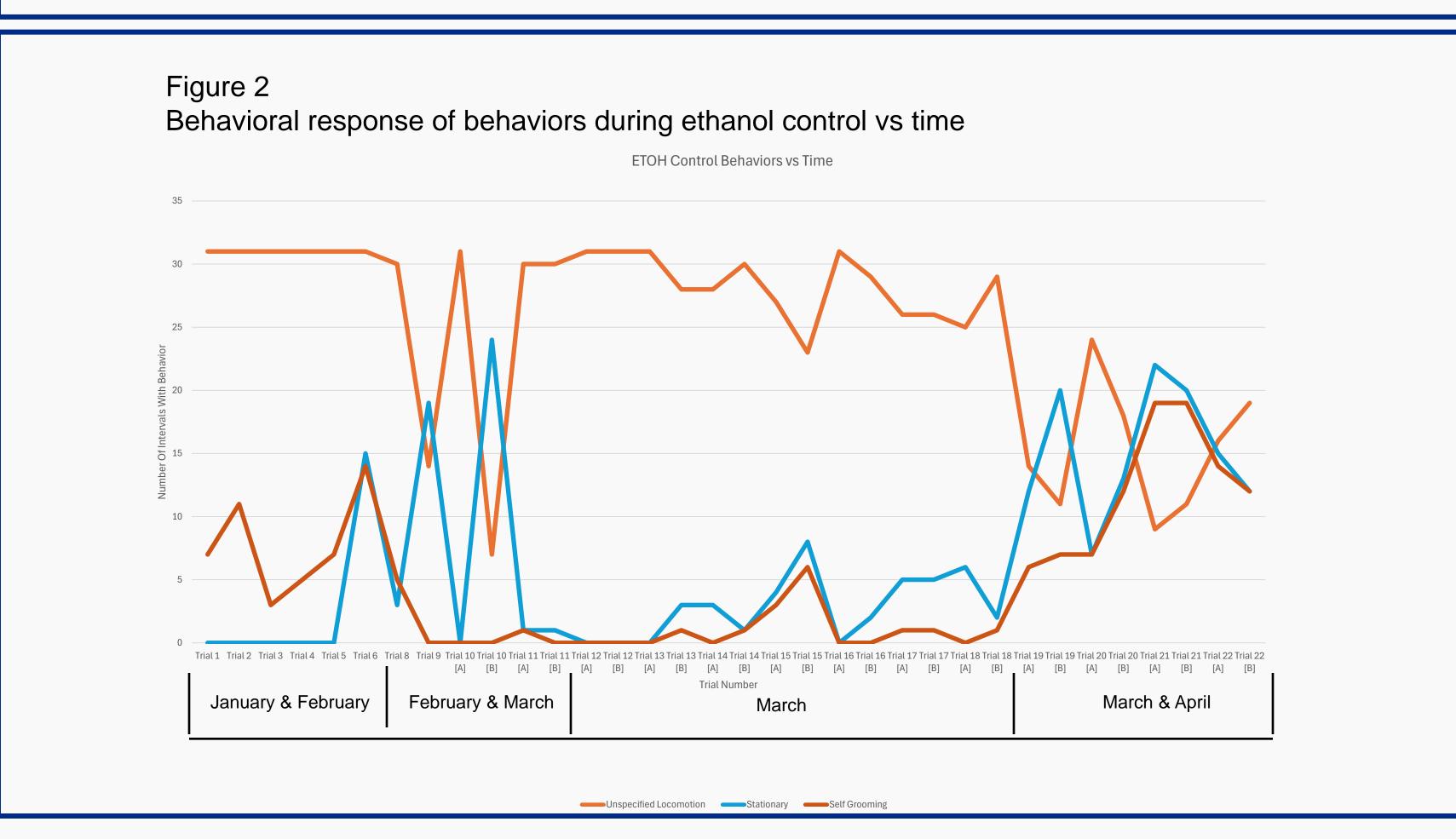
Lipid treatment behavioral experiments were performed using lipid solutions of linoleic acid, oleic acid, and a-linolenic acid diluted with ethanol. Worker caste ants were exposed to each lipid and an ethanol control. The ants' behavior was monitored at ten-second intervals for a total of five minutes.

Behavioral data was collected and tabulated using a behavioral ethogram. The data was then analyzed using a paired T-Test and visualized into a radar chart using the averaged behavioral data for each treatment.

In early April toward the end of the experiment, the A. texana queen perished. The ethanol control trials were used to visualize the timeline of behavior variations at different stages of the Atta texana colony's collapse.

Behavioral Experiments





Behavioral Ethogram

3 4 5

	Activity Scale		Description	
Α	Active	Aggressive	Lunging	Ant moves a short distance forward quickly, often seen with a biting motion
			Biting	Ant uses both mandibles to slice at the paper or plastic box
			Open mandibles	Ant has mandibles held open, seen while standing still and while moving
		Interest	Picking up paper	Using mandibles to lift the paper
			Touching paper	Ant stands on or walks over the paper
			Waving antennae toward paper	Ant walks in the direction of the paper while slowly lifting and moving antennae back and forth
Ina	Inactive		Locomotion	Ants move around the test container without a clear goal
		Stationary		Ants do not move from one point without movement of legs or antennae
lmi	ocial / munity sponses	Self-grooming		Repeatedly dragging antennae underneath forelegs, and then dragging forelegs through mouthparts
		Allogrooming		Ant will approach and be in contact with another ant while using its mouthparts to clean the other ants' cuticle
	Inspecting		ach other	Ant will face and use antennae to touch another ant

Discussion

This project revealed preliminarily statistically significant behavioral responses to the different lipid treatments. This incudes, a-Linolenic [Unspecified locomotion, stationary, mandibles open, waving antennae towards the paper, inspecting other ants, self-grooming]

Linoleic [Unspecified locomotion, stationary, mandibles open, waving antennae towards the paper, self-grooming]

Oleic [Mandibles open, waving antennae towards the paper, self-grooming]

These findings show a potential behavioral phenotype for each lipid treatment, suggesting that oleic acid, α-Linolenic acid, and linoleic acid may be used as allelochemicals in attine fungus-growing ants. Going forward, the next steps of this project would be to perform more replicates to determine if there is a true link between the lipid treatments and behavioral responses.

At the advent of the Queen A. texana ant perishing, the ethanol controls showed a potential increase of self-grooming and stationary behaviors. This may reveal a social-immunity based behavioral response that needs further study^{1, 2}.

References

¹Casillas-Pérez, Barbara, et al. "Dynamic Pathogen Detection and social feedback shape collective hygiene in ants." *Nature Communications*, vol. 14, no. 1, 3 June 2023, https://doi.org/10.1038/s41467-023-38947-y.

²Cotazo-Calambas, K. M., et al. "Behavioral response of the leaf-cutting Ant Atta Cephalotes (hymenoptera: Formicidae) to trichoderma sp.." *Journal of Insect Behavior*, vol. 35, no. 4, July 2022, pp. 92–102, https://doi.org/10.1007/s10905-022-09800-9.

³Garrett, Ryan W., et al. "Leaf processing behaviour in atta leafcutter ants: 90% of leaf cutting takes place inside the nest, and ants select pieces that require less cutting." *Royal Society Open Science*, vol. 3, no. 1, Jan. 2016, p. 150111, https://doi.org/10.1098/rsos.150111.

⁴Gómez-Díaz, Juan Sebastián, et al. "Hygienic behavior and antimicrobial peptide expression of the leaf-cutting Ant Atta Cephalotes (hymenoptera, Formicidae) to Metharhizium Anisopliae." *Journal of Hymenoptera Research*, vol. 91, 30 June 2022, pp. 335–356, https://doi.org/10.3897/jhr.91.82381.

⁵Khadempour, Lily, et al. "From plants to ants: Fungal modification of leaf lipids for nutrition and communication in the leaf-cutter ant fungal garden ecosystem." *mSystems*, vol. 6, no. 2, 27 Apr. 2021, https://doi.org/10.1128/msystems.01307-20.

⁶ Scientific Image and Illustration Software." *BioRender*, www.biorender.com/. Accessed 22 Mar. 2025.