8-2021

Distribution and ecological effects of Azteca Chartifex/Trigona in the Barro Colorado Nature Monument.

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Recommended Citation

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DISTRIBUTION AND ECOLOGICAL EFFECTS OF *AZTECA CHARTIFEX/TRIGONA* IN THE BARRO COLORADO NATURE MONUMENT

By

Rachel LeAnn Wells
B.A., Hendrix College, 2016

A Dissertation
Submitted to the Faculty of the
College of Arts and Sciences of the University of Louisville
in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy
in Biology

Department of Biology
University of Louisville
Louisville, Kentucky

August 2021

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A Dissertation Approved on

July 27, 2021

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DEDICATION

TO MOM, DAD, AND ROB:

For showing me every day what it means to be passionate, dedicated, and unique;
and for your unwavering love and support.

TO DR. MATTHEW D. MORAN:

Whose fault it is that I study ants. Thank you for starting me on this journey with a simple question and for always finding ways to facilitate your students' passions for science.

TO ROBERT ANTHONY SKOLIK:

For everything.
I would never have completed this journey without you.

ACKNOWLEDGEMENTS

I thank Noah Gripshover, Kane Lawhorn, Daniella Prince, and Aspen Workman for field assistance; Jeffrey Burchfield, Cesar Gutierrez, and Riley Kneale for technical assistance; Max Adams, Lindsey Barnes, Jelena Bujan, Evan Gora, Kane Lawhorn, Abby Nienaber, Daniella Prince, Jeannine Richards, Drew Seiler, and Heather Short for comments and guidance that improved each chapter tremendously; I thank the National Science Foundation for funding.

I thank my advisor, Steve Yanoviak, for his patience, guidance, and support over the years, even when I barely trudged along. I additionally thank my advisory committee, Rachelle Adams, Natalie Christian, Perri Eason, and Sarah Emery, for their input, support, and understanding throughout this process. I thank Melissa Cano and the STRI staff for logistical support and Paul DeMarco, Doris Meadows, and Terri Norris for showing much patience while helping me manage my funds throughout this process.

This work would not have been possible without the support and friendship of all of the Yanoviak lab members I have encountered along the way. I am indebted to each of you for the many hours of science and laughs over beers, karaoke nights, and much food. I am especially grateful to Max Adams for sharing his knowledge of canopy ants and tree climbing, Jelena Bujan for our sporadic coffee writing sessions, Jeffrey Burchfield for his constant support and shared love of Shania Twain, Evan Gora for answering my every question-especially those pertaining to stats, and Daniella Prince for always challenging me to be better. To everyone I have spent time and laughs with on BCI or in the states, thank you - with special thanks to Will Barker for our countless conversations that helped make me a more open person, Bianca Craig for all of the random and much-needed phone

conversations, and to Blake Cooper and Graham Senor for keeping me virtually social during a global pandemic.

Thank you to my parents - your love and guidance have facilitated everything that I have accomplished, and I can never thank you enough for all that you do. To my brother - thank you for always being there for me to look up to and for always letting me follow in your footsteps. Finally, thank you Robert Skolik for your endless support-for never doubting me, always encouraging me, and dancing crazily with me.

ABSTRACT

DISTRIBUTION AND ECOLOGICAL EFFECTS OF *AZTECA CHARTIFEX/TRIGONA* IN THE BARRO COLORADO NATURE MONUMENT

Rachel L. Wells

27 July 2021

This dissertation explores the behavior and ecology of a conspicuous and behaviorally dominant ant species in the tropical rainforest of Panama. Competition with aggressive species is an important factor that shapes local community structure. Eavesdropping on the chemical communication systems of such behaviorally dominant species can help heterospecific species to avoid negative interactions. I review the chemical communication system and known ecological effects of the Neotropical canopy ant, *Azteca chartifex/trigona* (Chapter 1). There are over 40 known compounds produced by *Azteca* workers, yet how these compounds impact heterospecific species is unclear in many circumstances. I assessed if heterospecific species respond to *A. trigona* pheromones by exposing workers of 29 canopy ant species to *A. trigona* alarm pheromones (Chapter 2). Seven species showed distinct responses to *A. trigona* pheromones and responses were not associated with phylogeny. The pheromones produced near ant nests may additionally be a reliable source for eavesdropping species and I used open-air sampling techniques to determine whether the air space surrounding *A. trigona* carton nests has a distinct chemical composition (Chapter 3). The air around disturbed *A. trigona* nests had higher concentrations of compounds associated with worker alarm pheromones, whereas undisturbed nests were chemically indistinguishable from the surrounding forest air. *Azteca trigona* workers aggressively outcompete for some resources and I experimentally assessed the effects of *A. trigona* on the composition of resident and colonizing ants by installing artificial nests in 28 tree crowns (Chapter 4). The presence of *A. trigona* did not affect the colonization frequency of artificial nests nor species composition of the resident ants in a tree; however, species composition of nest occupants differed between trees and nests located within the foraging territories of *A. trigona* were colonized less frequently. Finally, I determined if *A. trigona* was affected by landscape scale factors, including liana presence and canopy height (Chapter 5). *A. trigona* nests were more frequent in taller trees within older forests and had smaller colony sizes in liana removal plots. Collectively, the results summarized in this dissertation improves our understanding of canopy ant interactions and distributions in a tropical forest.

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CHAPTER I

INTRODUCTION

Communication is an essential component to the success of many animal species. Animals communicate using a variety of signaling modalities (e.g., visual, mechanical, chemical) to exchange information with nestmates, conspecifics, and heterospecifics (Endler 1992; Bradbury and Vehrencamp 2011). The specific information caried by signals is similarly diverse, such as warning of the presence of a predator or indicating the location of an essential resource (Wiley 1983). The success of a given signal reaching an intended receiver depends mainly upon its modality and the environment through which it is transmitted (Endler 1992). Signaling is costly to individuals as communicating is often energetically expensive and can reveal the location of the signaler or receiver. The potential costs of communication should act as a strong selective pressure that influences how animals communicate with each other (Endler 1993).

One major vulnerability of communicating is the potential for non-intended receivers, or eavesdroppers (Peake 2005), to detect and use the information contained in signals to their own advantage (Haynes and Yeargan 1999). For example, some species eavesdrop on vibrations, displays, and pheromones to locate prey, while other species eavesdrop on similar signals to usurp resources from interspecific competitors (Bernal et al. 2007, Suraci et al. 2017, Mihailova et al. 2018, Uetz et al. 2019, Virant-Doberlet et al. 2019). Signals that persist for long periods in the environment or can be detected over long distances can be especially reliable cues for eavesdroppers (McGregor 1993). In particular, chemical signals are susceptible to eavesdropping because they commonly are dispersed, and signalers have no control over the distribution or degradation of the compounds once they are emitted (Wyatt 2014). Moreover, diffusion gradients can reveal the location of a signaler, and this is especially problematic for species with signals that are maintained around a resource that is fixed in space and time (Bossert and Wilson 1963).

The nests of social insects often are stationary and can persist for many years (Hughes et al. 2008). Given the variety of pheromones associated with social insect activities (and thus occurring in close proximity to their nests), eavesdropping on these insects is expected to be very common (Billen 2006, Slaa and Hughes 2009, Leonhardt et al. 2016). Specifically, social insects use pheromones to establish and maintain foraging trails, recruit workers for nest defense, and to mark the nest location and its boundaries, among other behaviors (Blum and Brand 1972, Billen and Morgan 1998, Yew and Chung 2015). Many predators, parasitoids, and competitors eavesdrop on various social insect pheromones (van Zweden and d'Ettorre 2010, Lichtenberg et al. 2011, Mathis and Philpott 2012, Wen et al. 2017a, 2017b); however relative to eavesdropping on other signal modalities, these chemical eavesdropping interactions are understudied. Such interactions can have important ecological consequences, for example, when they affect the behaviors and distributions of coexisting species (Goodale et al. 2010).

Among social insects, the chemical communication systems of ants (Hymenoptera: Formicidae) are relatively well known, making ants an ideal taxon for investigations of chemical eavesdropping (Blum 1969, Attygalle and Morgan 1984, Jackson and Morgan 1993, Hefetz 2007). An individual ant worker contains an average of seven different glands, each producing compounds that can individually or collectively function as pheromones (Jackson and Morgan 1993; Billen 2009). A variety of ant associates eavesdrop on these different signals to find ants and their associated resources (reviewed by Adams et al. 2020) or to avoid potentially negative interactions with competitors (Figure 1). In particular, many species eavesdrop on ant trail pheromones, especially among co-occurring ants (Table 1).

However, the behavioral and ecological consequences of these interactions, and their frequency, are unknown in most cases. Uncovering these patterns requires identifying ant eavesdroppers and quantifying the effects of eavesdropping interactions, especially on species that are predicted to be most at risk to chemical eavesdroppers (i.e., species with large, long-lived colonies that maintain extensive trail networks around exposed nests; Adams et al. 2020).

The carton nests of some *Azteca* ant (Dolichoderinae) species are expected to be at high risk from many eavesdroppers. Carton-nesting *Azteca* species build large, exposed, free-hanging nests that can contain thousands of entrances and over 500,000 workers (Figure 2; Wheeler 1986). *Azteca* workers defend extensive trail systems and territorial boundaries via well-developed chemical defense systems (Adams 1994, Dejean et al. 2007), the components of which are so strong that humans can detect them when released (RW, *pers. obs.*). *Azteca* are often one of the most abundant ant species in a tree crown (Tobin 1995, Davidson 1997, Wilkie et al. 2010) influencing canopy arthropod distributions and access to resources, and such effects are mediated in part through chemical eavesdropping (Adams 1990, Yanoviak and Kaspari 2000, Dejean et al. 2007, Perfecto et al. 2014, Adams et al. 2019, Achury et al. 2020). Additionally, due to their large colony sizes and aggressive workers, *Azteca* often have cascading effects on canopy arthropods and their nesting tree (Table 2). Although over 50 compounds have been identified from *Azteca* workers, few studies have examined how *Azteca* chemistry influences these interactions.

In terms of chemical composition, the best-studied *Azteca* pheromones are those associated with defense and trail markings, both of which are produced by worker pygidial glands (Table 3)[1]. *Azteca* defensive compounds are composed of a variety of ketones and

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[1] Dolichoderine defense systems, relative to other ant subfamilies, are highly developed around chemical communication (Shattuck 1995). The pygidial gland in dolichoderine workers is greatly enlarged to facilitate this defense system (Blum 1969, Billen 1986). The pygidial gland is so much larger than the

iridoids. Ketones attract workers to potential threats, while iridoids enact aggressive defensive behaviors, such as mandible flaring and gaster-flagging (Blum 1969, Do Nascimento et al. 1998). The most commonly reported ketones from *Azteca* glands are 2-Heptanone and 2-Acetyl-3-methylcyclopentene, both of which are used by eavesdropping predators and parasitoids (Mathis et al. 2011, Mathis and Tsutsui 2016a). Although iridoids are likely also used by eavesdropping species, the specific isomers of these compounds remain unclear in many circumstances and no studies have experimentally tested their effects on potential eavesdroppers (Table 3). The compounds that elicit trail following behaviors are mainly composed of aldehydes such as 2-formyl-3-methylcyclopentene-acetaldehyde. Trail pheromones are exploited by some eavesdropping species, although it remains unknown which specific compounds are targeted (Wilson 1965, Adams 1990). Other known *Azteca* compounds include multiple nestmate recognition compounds (cuticular hydrocarbons) that are exploited by eavesdropping parasitoid phorid flies (Mathis and Tsutsui 2016b), and presumably various other taxa that remain to be studied.

Carton-nesting *Azteca* ants are often numerically and behaviorally dominant species in tropical forests. They often provide mutualistic benefits to their host tree, such as decreasing insect pest abundance and increasing soil nutrient content, while negatively affecting the behaviors and distributions of other canopy arthropods (Table 2). Thus, understanding their effects on heterospecific arthropods should provide better insight into the factors that maintain species cooccurrence and local biodiversity in the forest canopy. Improving our understanding of these interactions is the primary goal of this dissertation. Specifically, my research explores the chemical, community, and population ecology of the *Azteca* species complex *Azteca chartifex/trigona* (hereafter, *Azteca trigona* for simplicity) to

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pygidial glands in other ant subfamilies that for years it was thought to be a separate gland, termed the "anal gland" (Hölldobler and Wilson 1990).

evaluate if and how this common species influences heterospecific canopy ants within the Barro Colorado Nature Monument (BCNM) in Panama.

This first chapter provides an introductory framework for the dissertation by summarizing the known chemical components of *Azteca* pheromones, their associated eavesdroppers, and the ecological effects that *Azteca* workers have on plants and canopy arthropods. In Chapter 2, I measure the behavioral responses of cooccurring heterospecific ants to *A. trigona* alarm pheromones, and determine whether eavesdropping species share volatile worker odor profiles. I show that multiple canopy ants across different subfamilies exhibit avoidance behaviors when exposed to *A. trigona* alarm pheromones and such behaviors apparently are not due to shared chemical profiles with *A. trigona*. Chapter 3 further explores *Azteca* chemical ecology by asking whether *A. trigona* nests have a distinct chemical odor plume that could also be used by eavesdroppers to reliably locate *A. trigona* colonies. Contrary to expectations, I found that *A. trigona* nests are chemically camouflaged; the air surrounding *A. trigona* nests is chemically similar to that of the surrounding forest unless nests become disturbed. Chapters 4 and 5 shift away from chemical ecology to larger-scale questions within community and landscape ecology. Specifically, the goal of Chapter 4 was to determine if the composition of resident and colonizing ants differs between trees containing *A. trigona* and trees lacking *A. trigona*. I found that the presence of *A. trigona* influences the composition of ants colonizing artificial nest resources, but does not affect the overall composition of ants residing in a given tree crown. Finally, in Chapter 5 I used ground-based surveys and existing GIS database resources to associate landscape-level variation in *A. trigona* nest density with selected biological, geological, and topographical factors. I found that *A. trigona* nest density varied predictably with liana presence, soil type, and the interaction between forest age and canopy height. Finally, chapter six summarizes and explores the implications of the results of these projects. Collectively, the research projects summarized in this dissertation advance our understanding of the ecology of a common ant species and its chemically-mediated effects on other species in a Neotropical forest canopy.

TABLES

Table 1. A list of ant species that eavesdrop on trail pheromones of distantly related ant hosts. *Nest sharing* = host and parasite occur within the nest; *Location* = principal location of nests and foraging trails; *References* = relevant citations obtained via structured Google Scholar searches [see Adams et al. (2020) for details]. See Appendix I for list of references.

Subfamily: Trail parasite species	**Host species (Subfamily)**	**Nest sharing**	**Nest/trail location**	**References**
Dolichoderinae:				
Dolichoderus debilis	*Crematogaster carinata* (Myrmicinae)	Yes	Canopy	28
Dolichoderus cuspidatus	*Polyrhachis ypsilon* (Formicidae)	No	Canopy	13
Formicidae:				
Camponotus beebi	*Azteca chartifex* (Dolichoderinae)	Yes	Canopy	29
Camponotus blandus	*Pseudomyrmex termitarius* (Pseudomyrmecinae)	Yes	Ground/termite nest	30
Camponotus femoratus	*Crematogaster limata* (Myrmicinae)	Yes	Canopy	28
Camponotus femoratus	*Crematogaster levior* (Myrmicinae)	Yes	Canopy	28
Camponous lateralis	*Crematogaster scutellaris* (Myrmicinae)	No	Dead wood (tree, log)	31; 32
Camponotus rufifemur	*Crematogaster modiglianii* (Myrmicinae)	No	Canopy	13
Camponotus saundersi	*Polyrachis ypsilon* (Formicidae)	No	Canopy	13
Camponotus vitreus	*Crematogaster* cf. *polita* (Myrmicinae)	No	Canopy/stems	33
Camponotus sp.	*Crematogaster inflate* (Myrmicinae)	No	Canopy	34
Camponotus sp.	*Crematogaster coriaria* (Myrmicinae)	No	Canopy/deadwood	33
Camponotus sp.	*Crematogaster* sp. (Myrmicinae)	No	Unknown/deadwood	35
Lasius niger	*Formica rufibarbis* (Formicinae)	No	Underground	26
Oecophylla longinoda	*Cataulachus guineensis* (Myrmicinae)	No	Canopy	36
Polyrachis rufipes	*Gnamptogenys menadensis* (Ectatomminae)	No	Underground	37

Polyrachis sp.	*Camponotus cylindrica* (Formicinae)	Yes	Canopy	38
Myrmicinae:				
Cephalotes maculatus	*Azteca trigona* (Dolichoderinae)	No	Canopy	39
Cephalotes specularis	*Crematogaster ampla* (Myrmicinae)	No	Canopy	40
Formicoxenus nitidulus	*Formica rufa pratensis* (Formicinae)	Yes	Mound/underground	41
Pogonomyrmex colei	*Pogonomyrmex rugosus* (Myrmicinae)	Yes	Ground	42

Table 2. Ecological effects of *Azteca* ants on nesting trees and insect presence. *Filled circles* = positive effect of *Azteca* presence, *open circles* = negative effect of *Azteca* presence, ***X*** = no effect, and *n/a* = no known effects. Species are categorized by the system in which they were studied (e.g., Agroforestry, *Cecropia* Myrmecophytes, Other) and effects were categorized based on measured responses (e.g., plant health, insect pest abundance, herbivory damage). Within a species, every negative or positive effect listed has an associated reference. See Appendix I for list of references.

Species	Plant Health	Insect Pest Abundance	Herbivory Damage	Associate Abundance	Plant Competition	Ant Competition	References
Agroforestry:							
Azteca barbifex	○	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	**43**
Azteca delpini	●	*n/a*	*n/a*	○	*n/a*	*n/a*	**44**
Azteca sericeasur	○ ●	○ **X**	○ **X**	●	*n/a*	○	**45-55**
Azteca sp.	*n/a*	○	*n/a*	*n/a*	*n/a*	*n/a*	**56**
Azteca trigona group	*n/a*	○	*n/a*	*n/a*	*n/a*	*n/a*	**57**
Cecropia Myrmecophytes:							
Azteca alfari	●	*n/a*	○	*n/a*	○	*n/a*	**58-59**
Azteca constructor	●	*n/a*	○	*n/a*	○	*n/a*	**60**
Azteca muelleri	●	○	○	*n/a*	*n/a*	*n/a*	**61-62**
Azteca spp.	●	○	○	*n/a*	*n/a*	*n/a*	**63-64**
Other Myrmecophytes:							
Azteca depilis	●	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	**65**
Azteca spp.	●	○	○	*n/a*	*n/a*	*n/a*	**66-71**
Other:							
Azteca sp.	*n/a*	*n/a*	*n/a*	*n/a*	*n/a*	○	**72-74**
Azteca trigona group	●	*n/a*	*n/a*	●	*n/a*	○	**72, 74-78**

Table 3. Known chemical compounds produced by *Azteca* ants. Values in cells represent references and are arranged in numerical order. References listed as superscripts denote that eavesdroppers use the listed compound to eavesdrop on the associated *Azteca* workers. All ketone, iridoid, and aldehyde compounds were detected from worker pygidial glands, except for compounds denoted with an asterisk (*); in such cases compounds were detected from mandibular glands. See Appendix I for list of references.

Compound	***Azteca alfari***	***Azteca chartifex***	***Azteca instabilis***	***Azteca* JTL020**	***Azteca nigriventris***	***Azteca sericeasur***	***Azteca* spp.**	***Azteca velox***
KETONES								
1-Acetyl-2-methylcyclopentane						79[79]		
Cis-1-acetyl-2-methylcyclopentane			80		80			80
2-Acetyl-3-methylcyclopentene	81		80		80			80
2-Heptanone	81	82				83[83]	84	
2-Methylcyclopentanone			80		80			80
2-Pentanone						83		
6-methyl-5-hepten-2-one		81, 82						
IRIDOIDS								
Cis-trans-Iridodial		81						
Unknown 1-5, *cis-trans*-Iridodial	81							
Trans-cis-Iridodial	81	81						
Trans-trans-Iridodial	81	81						
Isomer of Iridodial		82						
Isomer of Nepetalactol	81	81						
Isopulegol		81						
ALDEHYDES								
2-formyl-3-methylcyclopentane-acetaldehyde	81							
2-formyl-3-methylcyclopentene-acetaldehyde	81	81						

2-methyl-1-cyclopentene-carboxaldehyde		81			
2-(3-methylcyclopentyl)propionaldehyde	81				
Benzaldehyde					85*
CUTICULAR HYDROCARBONS[8]					
n-C21			86		
n-C22			86		
n-C23			86	86, 87	
13-MeC23			86		
3-MeC23			86	86	
n-C24			86		
13 and 15-MeC24			86		
3 and 7-MeC24			86		
n-C25			86	86, 87	
13 and 15 and 3-MeC25			86	86, 87	
n-C26- and 10- and 12- and 14-MeC26			86	86, 87	
n-C27			86	86, 87	
3-MeC27				87	
11- and 13-MeC27			86	86, 87	
6,16- and 8,15-diMeC27				86, 87	
10- and 12- and 13- and 14-MeC28				86, 87	
n-C29				86, 87	
MeC29				86, 87	
7,15- and 7,17-diMe29				86, 87	

FIGURE LEGENDS

Figure 1. Selected chemical eavesdropping enemies and associates of ants. Individuals are categorized by biological lifestyle (*Ant Associate*) and communication system (*Host Pheromone/Associate Cue).* Where biological lifestyle is unknown, ant associates are categorized as myrmecophiles. Different colored boxes represent associates from different orders (or families for ants, bees, and wasps) and numbers within boxes indicate the reference number. Information from this table is adapted from Adams et al. 2020. See Appendix I for list of references.

Figure 2. The pendulous carton nest of a large *Azteca trigona* colony. This nest is ca. 2.5 meters in length.

FIGURES

Figure 1.

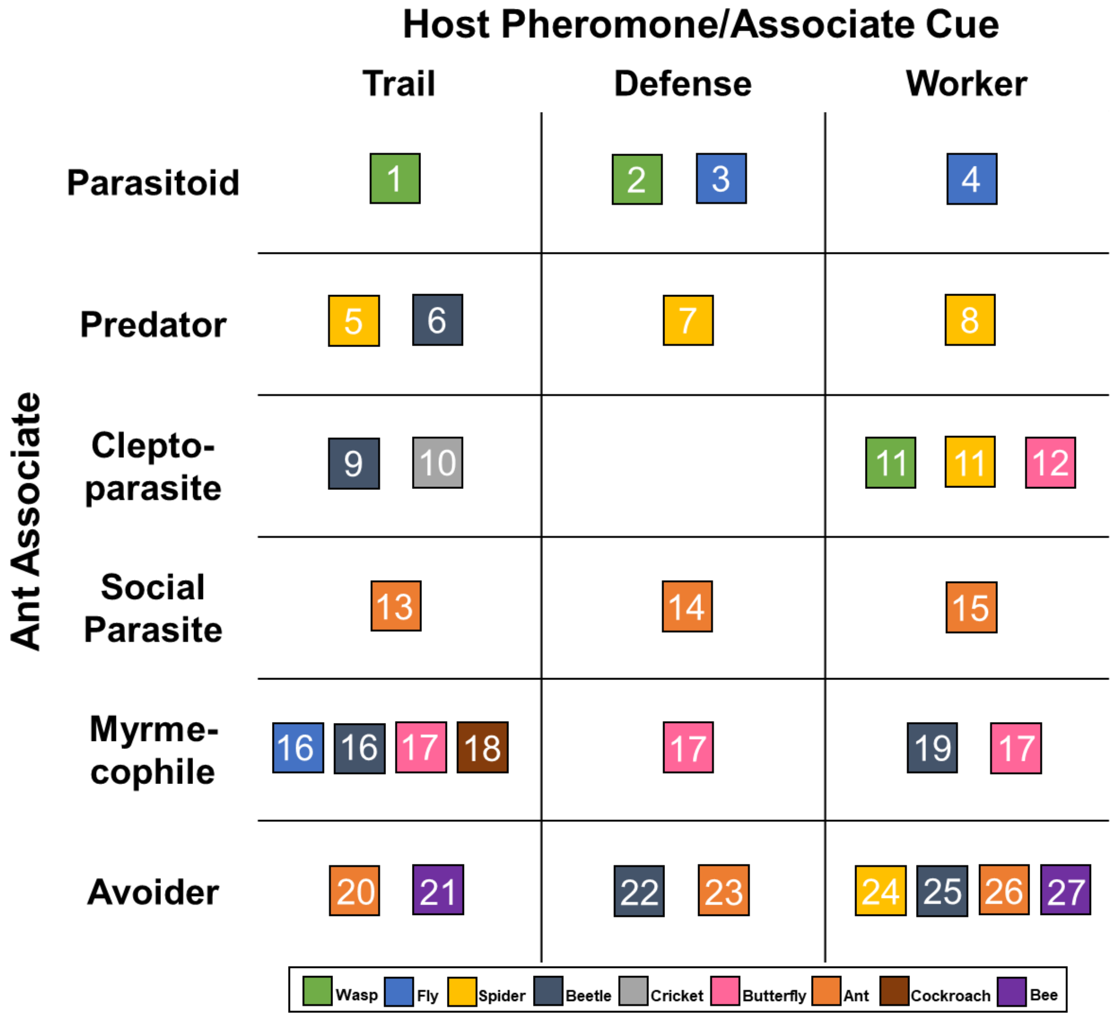


Figure 2.

CHAPTER II

EFFECTS OF *AZTECA TRIGONA* ALARM PHEROMONES ON HETEROSPECIFIC ANT BEHAVIORS

SUMMARY

Animals communicate with each other using a variety of signal modalities, any of which can provide useful information to non-intended receivers, or eavesdroppers. Eavesdropping on chemical signals is a widespread phenomenon, but its role in shaping the behavior of multi-species assemblages is poorly known. Here, I tested the hypothesis that workers of multiple Neotropical ant species change their behaviors when exposed to odors of the common canopy ant, *Azteca trigona*. I exposed workers of 29 canopy ant species (six subfamilies) to *A. trigona* alarm pheromones and compared their behavioral responses to the behavior of ants in control treatments (ambient air). Seven species showed distinct responses to *A. trigona* odors relative to the control. The most common behavioral responses were increased antennation and running. Chromatograms of worker volatile odors showed that the chemical profiles of *Atta colombica*, *Cephalotes atratus*, *C. basalis*, and *Dolichoderus bispinosus* share multiple peaks with *A. trigona* odors; however, the overall chemical composition differed interspecifically. The results of this study suggest that eavesdropping on heterospecific alarm signals allows ants to avoid generalized threats or negative interactions with aggressive *A. trigona* workers. Such eavesdropping presumably is selectively advantageous and may determine local arboreal ant species distributions and interspecific differences in access to resources in the forest canopy.

INTRODUCTION

Animals use a variety of signaling modalities (e.g., visual, mechanical, chemical) to communicate vital information to nestmates, conspecifics, and heterospecifics (Endler 1992, Wyatt 2014). Chemical signaling is widespread in nature, in part, because pheromones and other chemical messages often are relatively inexpensive to produce and can provide important contextual physiological and ecological information over long distances (Bossert and Wilson 1963, Symonds and Elgar 2008, Wyatt 2014). For insects, and especially social insects such as ants (Hymenoptera: Formicidae), chemical signaling is a ubiquitous part of life. Ants use a variety of compounds to organize individual behaviors and maintain colony structure (Roitberg and Isman 1992, Greenfield 2002, Heyman et al. 2017). Ants also leverage volatile chemical signals as alarm pheromones in part because they are detectable over long distances (Blum 1969, Lalor and Hughes 2011).

Alarm pheromones in some ant species occur as relatively conspicuous plumes of volatile compounds (Blum 1969; Attygalle and Morgan 1984). However, volatile chemical signals also can be problematic because the strength and distribution of the message cannot be controlled once it is emitted (Bossert and Wilson 1963). Such signals become freely available "public" information that is a reliable cue for exploitation by unintended receivers (i.e., eavesdroppers; Peake 2005) including ant-associated eavesdroppers, especially parasitoids and predators (Hölldobler and Wilson 1990; reviewed by Adams et al. 2020). Indeed, chemical eavesdropping is common in diverse groups of animals and is well-studied in insects (Stowe et al. 1995, Wyatt 2014). However, to our knowledge, no studies have explored the possibility that co-occurring ants eavesdrop on heterospecific ant alarm pheromones.

Interspecific eavesdropping on alarm pheromones is likely to be advantageous for co-occurring competitive or antagonistic species. Given that ant workers are valuable to their colonies directly as biomass (Wilson 1968) and indirectly via foraging and defensive behaviors (Carroll and Janzen 1973), selection should favor eavesdropping on any warning signals that consistently prevent worker loss by inducing avoidance behaviors in submissive species. Eavesdropping on heterospecific alarm pheromones could provide such a mechanism because alarm pheromones are reliable indicators of nearby dangers, including negative interspecific interactions among competing ants, the presence of predators and parasitoids, or any generalized threat or disturbance (Blum 1969, Lalor and Hughes 2011, Heyman et al. 2017) .

Azteca chartifex/trigona (Dolichoderinae) is a common Neotropical canopy ant species complex that has conspicuous nests (Figure 2), large polydomous colonies, pungent alarm pheromones, and aggressive workers (Wheeler 1986, Adams 1994, Longino 2007). Hereafter, I refer to this species complex as *A. trigona* for simplicity. In mangrove forests, *A. trigona* influences local ant community structure via territorial behaviors creating a mosaic of species distributions (Adams 1994). Alarm pheromones coordinate *A. trigona* defensive behaviors (Adams 1994), but the effects of *A. trigona* alarm pheromones on other ant species are unknown (Table 2).

Chemical components of alarm pheromones frequently are conserved within genera or subfamilies (Blum 1969, Wheeler et al. 1975, Du et al. 2019), thus eavesdropping species are likely to be closely related to the emitting species. Additionally, some behaviorally dominant ants competitively exclude other ant species that are ecologically or morphologically similar (Hölldobler and Wilson 1990, Andersen and Patel 1994, Adams 2016). If ants respond to the alarm pheromones of closely related heterospecific species, and if *A. trigona* similarly influences local species assemblages via competitive exclusion, I expect eavesdropping on *A. trigona* pheromones to be best developed in phylogenetically and behaviorally similar subordinate or codominant species. Various observations in the forests of Panama (e.g., Yanoviak and Kaspari 2000, Adams et al. 2017) indicate that most canopy ants avoid direct interactions with *A. trigona* workers while foraging. I also frequently observed strong negative behavioral responses (i.e., fleeing) by some arboreal species when presented with forceps contaminated with *A. trigona* alarm pheromones. These observations suggest that chemical cue recognition and eavesdropping on *A. trigona* is common among canopy ants.

The principal objective of this study was to determine if tropical canopy ants eavesdrop on the alarm pheromones of *A. trigona* ants. I focused on the following questions: Do ants change their behaviors when exposed to *A. trigona* alarm pheromones; if so, how do their behaviors change?; and are the behavioral changes phylogenetically constrained (i.e., are the types of behavioral responses more common in certain subfamilies)?. I also asked whether species with similar alarm pheromone composition to *A. trigona* are more likely to respond to *A. trigona* alarm pheromones. I predicted that differences in behavioral responses would be associated with ant subfamily identity, and species that are more phylogenetically similar to *A. trigona* would exhibit the greatest frequency of responses. I experimentally tested these predictions in Panama with freshly captured worker ants of 29 species.

METHODS

This study was conducted on Barro Colorado Island (BCI) in Panama between July 2016 and October 2018. BCI is a seasonally moist lowland forest with the wet season spanning May to December. All data for this study were collected during wet season months. More details about the site are provided elsewhere (Croat 1978, Leigh et al. 1996).

All worker ants used in experiments were collected by hand or with forceps from tree trunks and branches on BCI. Ants collected from a given colony were housed together in a vial until the start of an experiment, and all ants were used in experiments within 48 hours of collection. The 29 focal ant species (Table 3) were chosen to maximize phylogenetic diversity and to include species that commonly co-occur in trees with *Azteca trigona* at the study site (Adams et al. 2017).

Alarm Pheromone Trials

To determine which ant species responded to *A. trigona* alarm pheromones and how they responded, I placed a single ant (hereafter *recipient ant*) in a small glass arena (25.4 x 76.2 x 76.2 mm; Figures 3-4) and allowed it to acclimate for 3-5 minutes before each trial. At the beginning of each trial, volatile odors (hereafter *alarm pheromones*) were collected from >5 *A. trigona* workers by aggravating them inside a plastic vial (i.e., by shaking the vial) and then drawing air from the vial into the syringe. Twelve milliliters of air containing *A. trigona* alarm pheromones were then injected into the chamber via the syringe. Control treatments followed the same protocol using a new conspecific recipient ant worker for each trial and a clean syringe filled only with ambient air.

I recorded recipient ant behavior for multiple individual workers of 29 different species (Table 4). In each case, ant behavior was recorded five seconds before and five seconds immediately after exposure to *A. trigona* alarm pheromones using the video function of a compact digital camera (Canon-PowerShot ELPH 180, Canon Inc., Japan). I noted the following conspicuous changes in behavior of recipient ants: increased or decreased running speed, increased or decreased frequency of antennal movement, mandible flaring, and gaster flagging/tucking (i.e., positioning the gaster approximately orthogonal to the body axis either dorsally or ventrally; Curtis 1985, Obin and Vander Meer 1985; Figure 5). Behavioral responses of recipient ants in control treatments were similarly quantified. For four of the 29 species, I additionally tested worker behavioral responses to the alarm pheromones of the common species *Cephalotes atratus* and *Dolichoderus bispinosus* (Table 5).

Worker Odor Sampling

To compare alarm pheromone profiles among species, I collected "headspace" odor samples from workers of five focal ant species (2 dolichoderines, 3 myrmicines) in July 2016 (Table 4, Figure 6). Groups of five or more ants per species were placed into each of three clean 20 ml glass scintillation vials. A fourth empty vial was used as an ambient air control. Each of the four vials was capped with aluminum foil into which the beveled end of glass/teflon containing a tube chimney with ~30mg of PorapakQ was inserted. The four filters were connected to a single 6L/min, 12V diaphragm vacuum pump (Karlsson Robotics) via polypropylene tubing (Figure 6). Each collection lasted 30 minutes. After the collection period, volatile collection filters with ant odors were wrapped in aluminum foil and transported at ambient temperature back to the University of Louisville for extraction and GC/MS analysis.

GC/MS Analysis of Worker Odor Composition

Each volatile collection filter was eluted with 150 µl dichloromethane containing 10 ng μl^{-1} nonyl acetate as an internal standard (Rodriguez-Saona et al. 2001). Eluted samples were then analyzed with an Agilent 7890B Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA) in splitless mode with an inlet temperature of 250°C. The initial oven temperature was 35°C for sample injection and then increased 15°C per minute to 250°C with helium as a carrier gas at an average velocity of 22.5 cm s^{-1}. Samples were resolved on an Agilent DB-5 column (30 m length, 0.25 mm diameter, with a built-in 10 m DuraGuard pre-column). Volatile analytes were detected with an Agilent 5977A Mass Spectrometer with an EI ion source with the MS in scanning mode (50-550 m/z) and transfer line and ion source temperatures set at 230°C and 150°C. Peak areas within a sample were determined after peak deconvolution using the MassHunter software suite

(Agilent) to allow for individual peak area quantification. Compounds were identified based on comparison to the NIST14 spectral library, with spectral match thresholds for identified metabolites >80%. Afterwards, cross-sample alignments based on retention time were performed using the R package GCalignR (Ottensmann et al. 2018). For quantification, the concentration of each compound (ng min^{-1}) within a sample was calculated against the internal standard, and final analytical concentrations for each species were determined by subtracting analyte concentrations in the appropriate control sample from the corresponding ant worker samples.

Analyses

Analyses were performed in the R statistical environment (R Core Team 2019). I used Fisher's Exact tests to determine if the frequency of behavioral responses to *A. trigona* alarm pheromones differed from controls. A separate test was conducted for each species. For the four recipient species that were also tested against *Cephalotes atratus* and *Dolichoderus bispinosus* alarm pheromones, I performed pairwise comparisons using Fisher's Exact tests to evaluate differences in response frequency to each odor source (package RVAideMemoire; Hervé 2019). Bonferroni-adjusted alpha values were used for these post hoc tests to correct for multiplicity. I used a mixed-effect generalized linear model to compare subfamily responses to *A. trigona* and control odor treatments (*glmer*, package lme4). Responses of *A. trigona* workers to *A. trigona* and control odors were excluded from this analysis. The response term was a binomial variable (behavioral response or no response), subfamily and odor treatment were fixed effects, and species was a random effect. Stepwise model reduction with likelihood ratio tests removed the non-significant interaction between subfamily and odor source. To compare subfamily responses to *A. trigona* alarm pheromones and control odors separately, I used a glmer comparing subfamily responses using only recipient ants exposed to *A. trigona* alarm pheromones and a separate glmer with only recipient ants exposed to control odors. The response term was again a binomial variable, subfamily was the only fixed effect, and species was a random effect. I tested for differences between subfamily responses from each glmer test using a post-hoc Tukey HSD test.

I used a linear mixed effect model (*lmer*, package lme4) to determine whether the number of compounds per headspace sample varied by species. Species was treated as a fixed effect, and nest identity was a random effect for this analysis. I additionally compared the composition of compounds in the alarm pheromones among ant species using the R package *vegan* and the statistical software PRIMER. I used the concentration of each compound found in the samples for these analyses. The data were square-root transformed prior to calculating Bray-Curtis dissimilarity, and the distance matrix was used to generate a non-metric multidimensional scaling ordination (NMDS, R package *vegan*). I used PERMANOVA and the associated pair-wise analysis in PRIMER (version 6.1.18) to compare the composition matrix among the five ant species. I also compared beta diversity among species using a PERMDISP in PRIMER, which compares the magnitude of dissimilarity among the response types (Anderson et al. 2006). PERMDISP creates a derived centroid for each species and calculates the average sample deviation from the centroid. The average distance from the centroid is greater for factors that have higher β-diversity (i.e., species that have more variation in chemical composition among samples; Anderson et al. 2006). Finally, I performed an indicator species analysis (*indicspecies* package; De Cáceres and Legendre 2009) to identify compounds that were strongly associated with the different ant species (Dufrêne and Legendre 1997).

RESULTS

Seven of the 29 focal ant species responded more frequently to *A. trigona* odors than to ambient air (Figure 7). Although three of the seven species were dolichoderines, ants in all six focal subfamilies showed some behavioral response to *A. trigona* pheromones. Specifically, when worker responses were pooled at the subfamily level, response frequencies for *A. trigona* odors were consistently higher than for controls ($X^2 = 80.18$, $df = 1$, $p < 0.001$) and differed among subfamilies ($X^2 = 18.60$, $df = 5$, $p = 0.002$). Subfamilies responded differently to both control odors ($X^2 = 15.27$, $df = 5$, $p = 0.009$) and *A. trigona* alarm pheromones ($X^2 = 14.67$, $df = 5$, $p = 0.012$). Specifically, dolichoderines and formicines responded to control odors at a higher frequency than myrmicines and pseudomyrmecines while only dolichoderines responded to *A. trigona* pheromones at a higher frequency than myrmicines and pseudomyrmecines (Table 6, Figure 8).

Three of the four recipient species (*Atta colombica*, *Cephalotes umbraculatus*, and *Ectatomma tuberculatum*) exposed to multiple odor sources responded to the four odors (Table 7; Figure 9). *Atta colombica* responded more frequently to *A. trigona* odors than to controls, *C. umbraculatus* responded more frequently to *Dolichoderus bispinosus* odors than to controls, and *E. tuberculatum* responded more frequently to *A. trigona*, *D. bispinosus*, and *C. atratus* odors than to controls. *Crematogaster limata* responded similarly to all four odor sources.

In all, 382 individual ants (out of 982) changed their behaviors when exposed to pheromones or ambient air. Half (52%) of the 580 ants that were exposed to *A. trigona* pheromones showed a behavioral response, whereas only 24% of the 452 ants exposed to control odors responded. Most (77%) of the responding ants exhibited a combination of altered running speed and increased antennating frequency (Table 8, A+R). The least common behavioral change was gaster flagging, although dolichoderines exhibited combined behaviors that included gaster flagging in 26% of responses (Table 9).

The average (±SD) number of different compounds identified per sample was 27.8 ± 3.3 (n = 30 samples). The average number of different compounds detected did not differ among ant species ($X^2 = 6.37$, df = 4, $p = 0.173$); however, the composition of compounds differed among ant species (PERMANOVA: pseudo-$F_{4,25} = 3.29$, $p < 0.001$, Figure 10). Specifically, the composition of pheromone samples from *Atta colombica*, *Cephalotes atratus*, *C. basalis*, and *Dolichoderus bispinosus* differed from those of *Azteca trigona* (Tables 10-11). Beta-diversity in the compounds also differed among species, with overdispersion between samples within some species (PERMDISP: $F_{4,25} = 24.65$, $p < 0.001$). This difference in β-diversity could be attributable to uneven sample sizes among species and likely was driven by the *A. trigona* odors (Figures 10-11, Table 11). The indicator species analysis revealed five compounds associated with three species and only one unidentified compound associated with *A. trigona* (and *Dolichoderus bispinosus*; Table 12). Some of the most common compounds detected in this study were similarly associated with ants in other studies (Table 10).

DISCUSSION

Here I show that multiple Neotropical canopy ant species across a broad distribution of subfamilies change their behaviors when exposed to the alarm pheromones of the common and behaviorally aggressive species, *A. trigona*. Four of the six subfamilies tested included species that were both responsive and non-responsive to *A. trigona* pheromones (except for Ectatomminae, in which only one species was tested), suggesting that eavesdropping is a selective phenomenon among coexisting ant species that does not appear to be based on phylogenetic relatedness. Eavesdropping species occur across all domains of life (Stowe et al. 1995, Joint et al. 2007) and eavesdropping specifically on heterospecific alarm signals in animals is likely to be advantageous in many circumstances (Stowe et al.

1995, Adams et al. 2020). In this study, there was a rarity of aggressive responses to *A. trigona* odors (e.g., 77% of responders exhibited more frequent antennation and faster running speeds, which was the same percentage of response types to the control air), suggesting that avoidance of a potentially threatening species is the basis for eavesdropping behaviors among the focal ants. Such an "ecology of fear" occurs among many animal taxa (Pfeiffer 1962, Apfelbach et al. 2005, Goodale and Nieh 2012).

The results of this study do not support the prediction that species responding to *A. trigona* alarm pheromones would be closely related (i.e., dolichoderines) and have similar alarm pheromone composition. More than half of the responding species were non-dolichoderines, and characterization of volatile odors from five species suggest that *A. trigona* have a distinct alarm pheromone composition. Additionally, *Dolichoderus bispinosus* and *Atta colombica* shared two of the most concentrated compounds with *A. trigona*, although *A. colombica* behaviorally responded to *A. trigona* while *D. bispinosus* did not. The major components of alarm pheromones that elicit worker responses are often specific within ant subfamilies or genera (Blum 1969). For example, cyclopentanoid monoterpenes and sulcatone (6-methyl-5-Hepten-2-one) are known to occur only in *Azteca* alarm pheromones (McCann et al. 2013). These compounds elicit sustained alarm responses in *Azteca* workers and were found in our *A. trigona* headspace samples (Blum 1969, Wheeler et al. 1975, McCann et al. 2013). Although the most concentrated compound (on average) in the alarm pheromones of *A. trigona* and *D. bispinosus* was the same, *A. trigona* alarm pheromones did not elicit a response from *D. bispinosus* workers. Ants often only respond to pheromones with a specific chemical ratio (Blum 1969, Binz et al. 2014), suggesting that *D. bispinosus* either does not detect the odors of *A. trigona*, or ignores them. Additional research is needed to isolate the specific compound or compounds within the *A. trigona* alarm pheromone that elicit behavioral responses from heterospecifics.

The results of this study suggest that the responses of ant workers to *A. trigona* alarm pheromones are not phylogenetically based. Instead, such responses likely reflect ecological pressures as I found that ant workers from the same species respond at different frequencies to various heterospecific alarm pheromones. Given that ant workers are valuable to their colonies both directly and indirectly (Wilson 1968, Carroll and Janzen 1973) selection should favor behavioral response to interspecific alarm pheromones when such responses prevent worker loss. I hypothesize that the species responding to *A. trigona* in this study are those that are more likely to have negative interactions with *A. trigona* workers (i.e., competing species). For instance, *A. trigona* tend to have non-overlapping foraging territories with the responding species *A. colombica* and *Ectatomma tuberculatum* (Jutsum et al. 1981, Armbrecht et al. 2001). Additional field-based studies and natural history observations are needed to clarify links between pheromone eavesdropping and foraging decisions among potentially competing ants (Adams et al. 2020).

The results of this study support the hypothesis that workers of multiple potentially co-occurring ant species behaviorally respond to the alarm pheromones of *A. trigona*. This outcome is consistent with observations of eavesdropping in a variety of non-ant systems (Pfeiffer 1962, Goodale and Nieh 2012). However, the specific compounds that elicit responses, and the ecological consequences of responding to *A. trigona* alarm pheromones (e.g., potential loss of access to food resources) remain unknown. Understanding such patterns will clarify the role of chemical eavesdropping on species interactions, foraging behavior, and community structure in arboreal ants.

TABLES

Table 4. Summary of tests conducted on workers of 29 recipient ant species exposed to *Azteca trigona* alarm pheromones and ambient air (*Control*). Numbers indicate sample size; workers were used only once. * = headspace sample collected for pheromone composition analysis.

	Odor Source	
Recipient Species	*A. trigona*	Control
Dolichoderinae		
*Azteca trigona**	46	31
*Dolichoderus bispinosus**	44	47
Dolichoderus debilis	31	34
Dolichoderus laminatus	20	14
Ectatomminae		
Ectatomma tuberculatum	29	26
Formicinae		
Camponotus atriceps	6	5
Camponotus sericeiventris	31	30
Camponotus sp. 1	10	4
Camponotus sp. 2	21	19
Myrmicinae		
*Atta colombica**	37	37
*Cephalotes atratus**	35	25
*Cephalotes basalis**	29	27
Cephalotes christopherseni	9	6
Cephalotes grandinosus	4	2
Cephalotes maculatus	7	6
Cephalotes minutus	6	5
Cephalotes umbraculatus	24	16
Crematogaster acuta	13	11
Pheidole sp. 1	5	4
Ponerinae		
Neoponera foetida	7	2
Neoponera sp. 1	7	3
Neoponera verenae	8	10
Neoponera villosa	6	6
Pseudomyrmecinae		
Pseudomyrmex boopis	19	17
Pseudomyrmex elongatus	13	12
Pseudomyrmex gracilis	21	18
Pseudomyrmex oculatus	31	25
Pseudomyrmex rochai	4	5
Pseudomyrmex sp. 1	7	5

Table 5. Summary of source-recipient tests conducted on species exposed to four odor sources. Numbers indicate sample size. For *Tests*, *B* = species used in body size analysis, and *H* = headspace sample collected for pheromone composition analysis.

Recipient Species	**Odor Source**			
	A. trigona	*C. atratus*	*D. bispinosus*	Control
Ectatomma tuberculatum	29	34	27	26
Atta colombica	37	10	11	37
Cephalotes umbraculatus	24	17	27	16
Crematogaster limata	13	11	10	11

Table 6. Pairwise comparison of subfamily responses to *Azteca trigona* and control odor sources. Behavioral responses within subfamilies were compared to responses between each subfamily tested. Upper cell values are the z-values and lower cell values are the P-values from Tukey post hoc tests. *Doli.* = Dolichoderinae, *Ecta.* = Ectatomminae, *Form.* = Formicinae, *Myrm.* = Myrmicinae, *Pone.* = Ponerinae, *Pseu.* = Pseudomyrmecinae.

	Ecta.	**Form.**	**Myrm.**	**Pone.**	**Pseu.**
Doli.	-1.78 0.465	-1.20 0.828	-4.54 <0.001	-3.01 0.030	-4.42 <0.001
Ecta.		0.906 0.942	-1.07 0.890	-0.765 0.972	-1.31 0.773
Form.			-3.03 0.028	-2.00 0.331	-3.13 0.021
Myrm.				0.168 1.00	-0.486 0.996
Pone.					-0.495 0.996

Table 7. Pairwise comparisons between species responses (*recipient species*) to different odor sources (*odor sources compared*). Cell values are based on Fisher's Exact test p-values. Values in bold indicate significant differences based on Bonferroni adjusted alpha = 0.0125.

		Odor Sources Compared		
Recipient Species		***C. atratus***	***D. bispinosus***	**Control**
E. tuberculatum	***A. trigona***	0.548	0.183	**0.001**
	C. atratus		1.00	**<0.001**
	D. bispinosus			**<0.001**
A. colombica	***A. trigona***	1.00	1.00	**<0.001**
	C. atratus		1.00	0.031
	D. bispinosus			0.763
C. umbraculatus	***A. trigona***	0.119	0.033	1.00
	C. atratus		1.00	0.040
	D. bispinosus			**<0.001**

Table 8. The different combinations of qualitative behavioral responses (*Behavior*) observed in this study. Values under *A. trigona* and *Control* are the percentage of recipient ants that exhibited a given *Behavior*. *Total* = the total number of ants exhibiting a given *Behavior* (each ant was assigned to only one *Behavior*). *R* = changed running speed, *A* = changed antennation frequency, *M* = mandible flaring, *G* = gaster flagging (see Figure 8). In all, 530 ants were exposed to *Azteca trigona* alarm pheromones and 452 ants were exposed to control odors. Each ant was exposed to only one odor source.

Behavior	***A. trigona***	**Control**	**Total**
None	41	59	347
A	43	57	186
R	55	46	55
M	25	75	8
G	50	50	4
A + R	73	27	292
A + M	59	41	17
A + G	33	67	9
G + R	40	60	5
M + R	100	0	3
G + M	0	100	1
A + G + R	91	9	35
A + M + R	56	44	18
A + G + M	100	0	1
G + M + R	0	0	0
A + G + M + R	0	100	1

Table 9. Different combinations of behavioral responses to *Azteca trigona* alarm pheromones observed among ant subfamilies. See Table 6 for explanation of *Behavior* abbreviations. See Table 4 for subfamily abbreviations.

Behavior	**Doli.**	**Ecta.**	**Form.**	**Myrm.**	**Pone.**	**Pseu.**
A + R	65	17	29	56	9	38
A + M	3	0	5	1	1	0
A + G	2	1	0	0	0	0
G + R	1	0	0	1	0	0
M + R	1	0	1	1	0	0
A + G + R	24	0	2	6	0	0
A + M + R	6	0	2	1	1	0
A + G + M	1	0	0	0	0	0
Total Respond	**103**	**18**	**39**	**66**	**11**	**38**
Total Tested	**141**	**29**	**68**	**169**	**28**	**95**

Table 10. The most abundant compounds detected in the alarm pheromone samples of five ant species. Values under *A. trig*, *D. bisp*, *A. colo*, *C. atra*, and *C. basa* are average concentrations (ng min^{-1}). *Compound Name* = NIST14 library match, *Retention Time* = GC retention time (min), *% Name Certainty* = NIST14 spectral match confidence (> 80%). * = compounds associated with a given ant species based on indicator species analysis.

Compound Name	**Retention Time**	**% Name Certainty**	***A. trig***	***D. bisp***	***A. colo***	***C. atra***	***C. basa***
UNKNOWN 1	5.791	n.a.	72.2*	64.9*			
2-(1-Methylcyclopropyl)aniline	10.494	95	17.8	3.4		3	
N,N-dibutyl-Formamide	10.157	81	12.8				
n-Decanoic acid	10.537	80	10.5		2.1		
N,N-diethyl-4-methyl-Benzamide	12.609	96	9.9		29		164.8
6-methyl-5-Hepten-2-one	6.826	85	7				
Naphthalene	9.113	94		8.6*	*	3*	9*
UNKNOWN 2	6.585	n.a.		4.3*	2.7*		2.5*
1,1'-oxybis-Heptane	7.254	80		4.3*			
D-Limonene	7.358	94		3	9.8*		5.7*
UNKNOWN 3	7.399	n.a.		2.5	1.9		
3-Carene	6.339	90		2.4			
Decanal	9.121	84			4.1*		
Nonanoic acid	9.613	84		*	2.4*	*	
Toluene	4.591	91			2		
UNKNOWN 4	4.806	n.a.				6.7	
1,3-dimethyl-Benzene	5.618	92				6.3	9.8

Benzaldehyde	6.629	89		5.5	
4-methylene-1-(1-methylethyl)-Cyclohexene	6.866	89		5.4	
UNKNOWN 5	9.173	n.a.	*	3.2*	
Caryophyllene	11.383	94		3	1.6
Phenol	6.690	84		3	1.5
UNKNOWN 6	5.750	n.a.			33.8*
Phenylethyl Alcohol	8.231	92			8.3
N,N,3-trimethyl-Benzamide	12.188	96			2.7*

Table 11. Pairwise comparisons of the odor compositions of five focal ant species. Numbers are p-values from post-hoc tests; bold values indicate significantly different chemical profiles.

	A. colo	**C. atra**	**C. basa**	**D. bisp**
A. trig	**<0.001**	**0.001**	**<0.001**	**0.004**
A. colo		0.102	0.100	0.096
C. atra			0.105	0.107
C. basa				0.102

Table 12. The list of indicator compounds from alarm pheromone headspace samples taken from five ant species. Values are the indicator value (IndVal) and adjusted p-values (P).

Species	**Compound Name**	**IndVal**	**P**
A. colo	Decanal	0.821	0.036
C. basa	N,N,3-trimethyl-Benzamide	0.816	0.018
C. basa	UNKNOWN 6	0.811	0.047
D. bisp	UNKNOWN 7	0.976	0.004
D. bisp	1,1'-oxybis-Heptane	0.88	0.009
A. colo & *C. atra*	UNKNOWN 8	0.915	0.003
A. colo & *C. atra*	UNKNOWN 5	0.903	0.004
A. colo & *C. atra*	1-(4-ethylphenyl)-Ethanone	0.867	0.013
A. colo & *C. atra*	UNKNOWN 9	0.812	0.027
C. atra & *D. bisp*	1,2,4-trimethyl-Benzene	0.964	0.001
A. colo & *C. basa*	bis(2-methylpropyl) ester 1,2-Benzenedicarboxylic acid	0.868	0.019
A. colo & *C. basa*	D-Limonene	0.816	0.009
A. colo & *D. bisp*	UNKNOWN 10	0.896	0.016
A. trig & *D. bisp*	UNKNOWN 1	1	0.001
A. colo, *C. atra*, & *D. bisp*	Nonanoic acid	0.979	0.001
A. colo, *C. atra*, & *D. bisp*	2-phenoxy-Ethanol	0.908	0.008
A. colo, *C. atra*, & *D. bisp*	Hexamethyl-Cyclotrisiloxane	0.884	0.004
A. colo, *C. atra*, & *D. bisp*	Acetophenone	0.881	0.012
A. colo, *C. atra*, & *D. bisp*	UNKNOWN 11	0.877	0.008
A. colo, *C. basa*, & *D. bisp*	UNKNOWN 12	0.902	0.003
A. colo, *C.atra*, *C. basa*, & *D. bisp*	Naphthalene	0.986	0.001
A. colo, *C.atra*, *C. basa*, & *D. bisp*	Vanillin	0.913	0.004

FIGURE LEGENDS

Figure 3. Diagram of the experimental arena used for behavioral tests. The rectangular chamber is constructed from 25.4 x 76.2 x 76.2 mm glass microscope slides secured with hot-melt adhesive. Recipient species were individually placed in the chamber and exposed to source odors via a syringe.

Figure 4. Experimental arena with a *Camponotus sericeiventris* worker acclimating to the arena (a), antennating in response to *Azteca trigona* odors (b), and running to the odor source (c).

Figure 5. The stereotypical ant responses to odors observed in this study: running (a), antennating (b), mandible flaring (c), and gaster flagging (d, e).

Figure 6. Diagram of the headspace sampling set-up. a) Scintillation vial containing worker ants with tubing and filter attachment. b) Wires (dark green lines) were attached from the two pumps (red dots) to the battery (rectangle) and plastic tubing from the pumps connected the four filters to the scintillation vials with at least five worker ants (#) and the empty vial (*)

Figure 7. The proportion (with ± 95% Clopper-Pearson CI) of trials in which workers of 29 recipient ant species exhibited behavioral responses when exposed to the alarm odors of *Azteca trigona* (black bars) and ambient air controls (gray bars). *Ecta*. = Ectatomminae. Significant differences are indicated as * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Figure 8. The proportion (with ± 95% Clopper-Pearson CI) of behavioral responses of five recipient ant subfamilies exposed to odors from *Azteca trigona* (filled bars) and ambient air (open bars). Numbers above bars represent sample sizes. Lowercase letters indicate proportions that do not differ; ab letters indicate differences from *A. trigona* odors whereas xy letters indicate differences from control odors.

Figure 9. The proportion (with ± 95% Clopper-Pearson CI) of behavioral responses of four recipient ant species exposed to odors from four different source ant species: *Azteca trigona* (black bars), *Cephalotes atratus* (dark grey bars), *Dolichoderus bispinosus* (light grey bars), and ambient air (white bars). Significant differences in mean proportion among odor sources within a recipient ant species are indicated by different letters. *Azteca trigona* and ambient air information also in Figure 7.

Figure 10. Non-metric multidimensional scaling ordination of the alarm pheromone chemical composition for five focal ant species. Ellipses are the 95% confidence intervals surrounding the centroid of each group. A. trig = *Azteca trigona*, D. bisp = *Dolichoderus bispinosus*, A. colo = *Atta colombica*, C. atra = *Cephalotes atratus*, and C. basa = *C. basalis*. Stress = 0.198.

Figure 11. Representative chromatograms of the odor profiles of *Azteca trigona* compared to four other ant species. The *A. trigona* plot shows the control (grey) and *A. trigona* (black) chromatogram; controls were excluded from the other four chromatograms for clarity. Black arrows indicate peaks that are shared with *A. trigona* odors and that were not masked by the control. Red arrows represent the internal standard.

FIGURES

Figure 3.

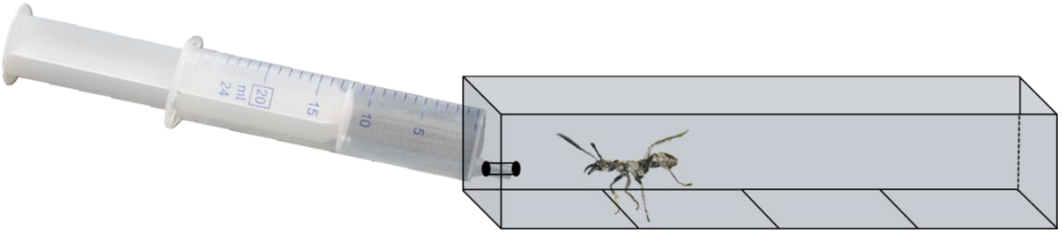


Figure 4.

Figure 5.

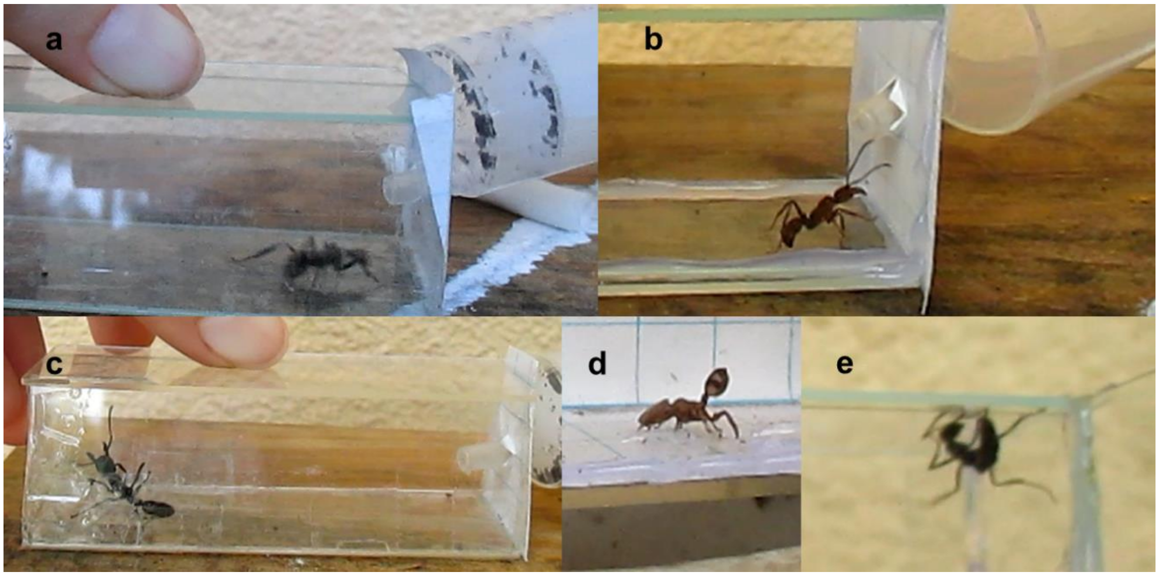


Figure 6.

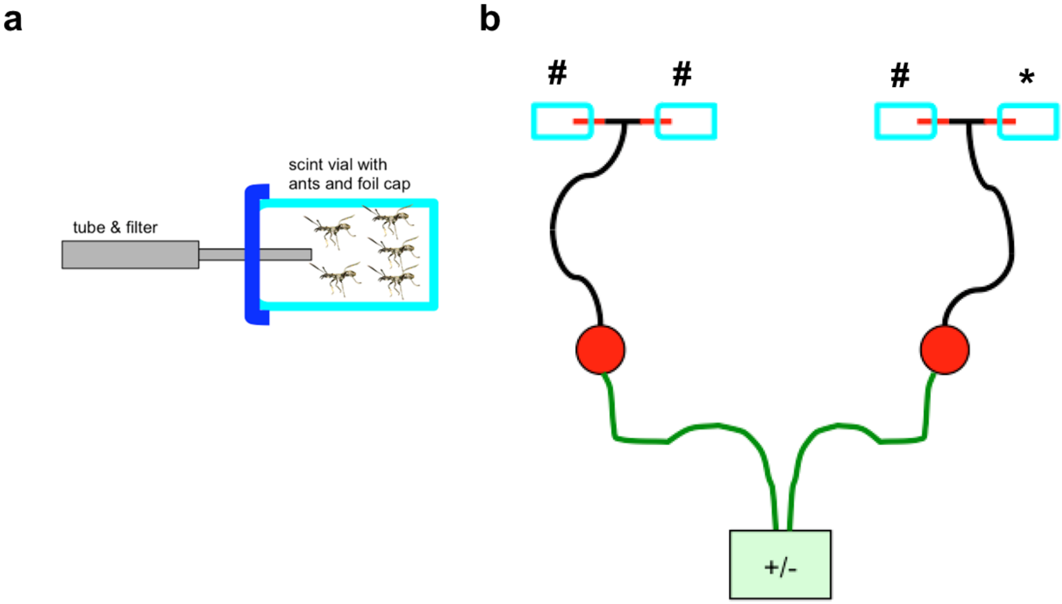


Figure 7.

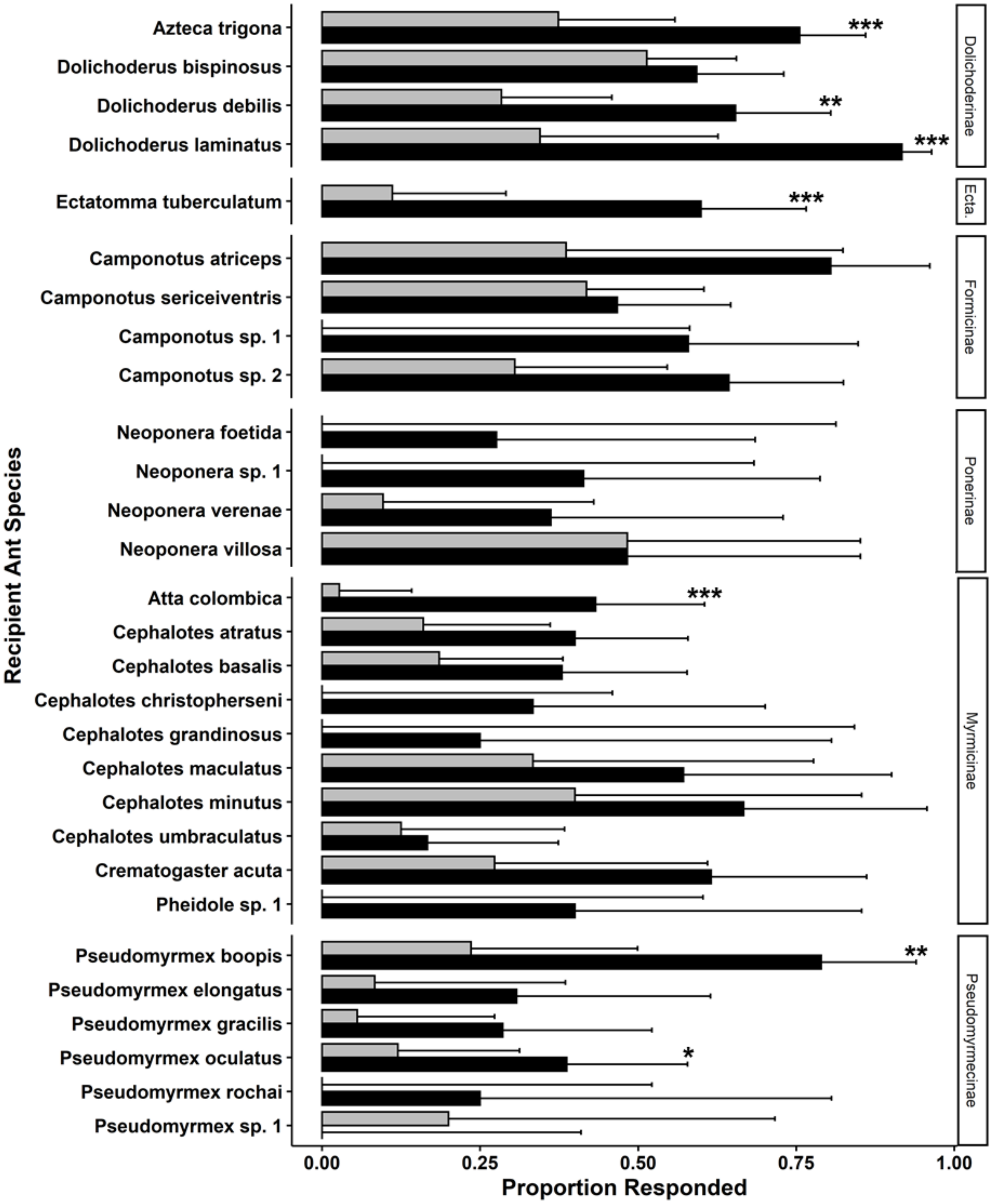

Azteca trigona
Dolichoderus bispinosus
Dolichoderus debilis
Dolichoderus laminatus
Ectatomma tuberculatum
Camponotus atriceps
Camponotus sericeiventris
Camponotus sp. 1
Camponotus sp. 2
Neoponera foetida
Neoponera sp. 1
Neoponera verenae
Neoponera villosa
Atta colombica
Cephalotes atratus
Cephalotes basalis
Cephalotes christopherseni
Cephalotes grandinosus
Cephalotes maculatus
Cephalotes minutus
Cephalotes umbraculatus
Crematogaster acuta
Pheidole sp. 1
Pseudomyrmex boopis
Pseudomyrmex elongatus
Pseudomyrmex gracilis
Pseudomyrmex oculatus
Pseudomyrmex rochai
Pseudomyrmex sp. 1
***
**
***
***
***
**
*
Dolichoderinae
Ecta.
Formicinae
Ponerinae
Myrmicinae
Pseudomyrmecinae
Recipient Ant Species
0.00
0.25
0.50
0.75
1.00
Proportion Responded

Figure 8.

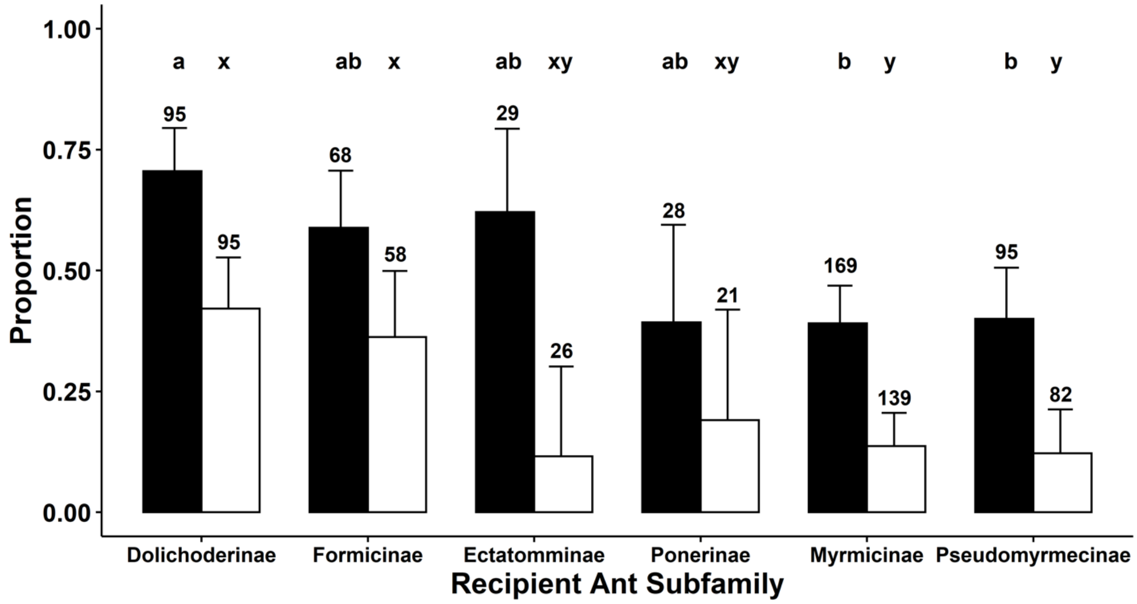


Figure 9.

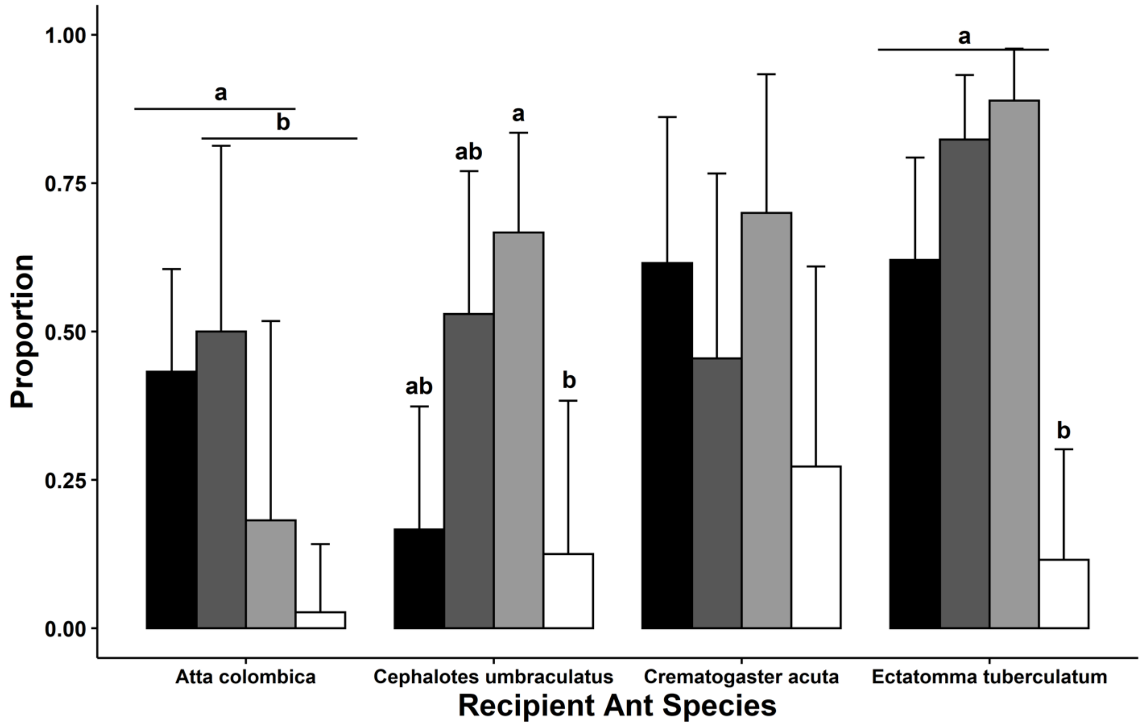
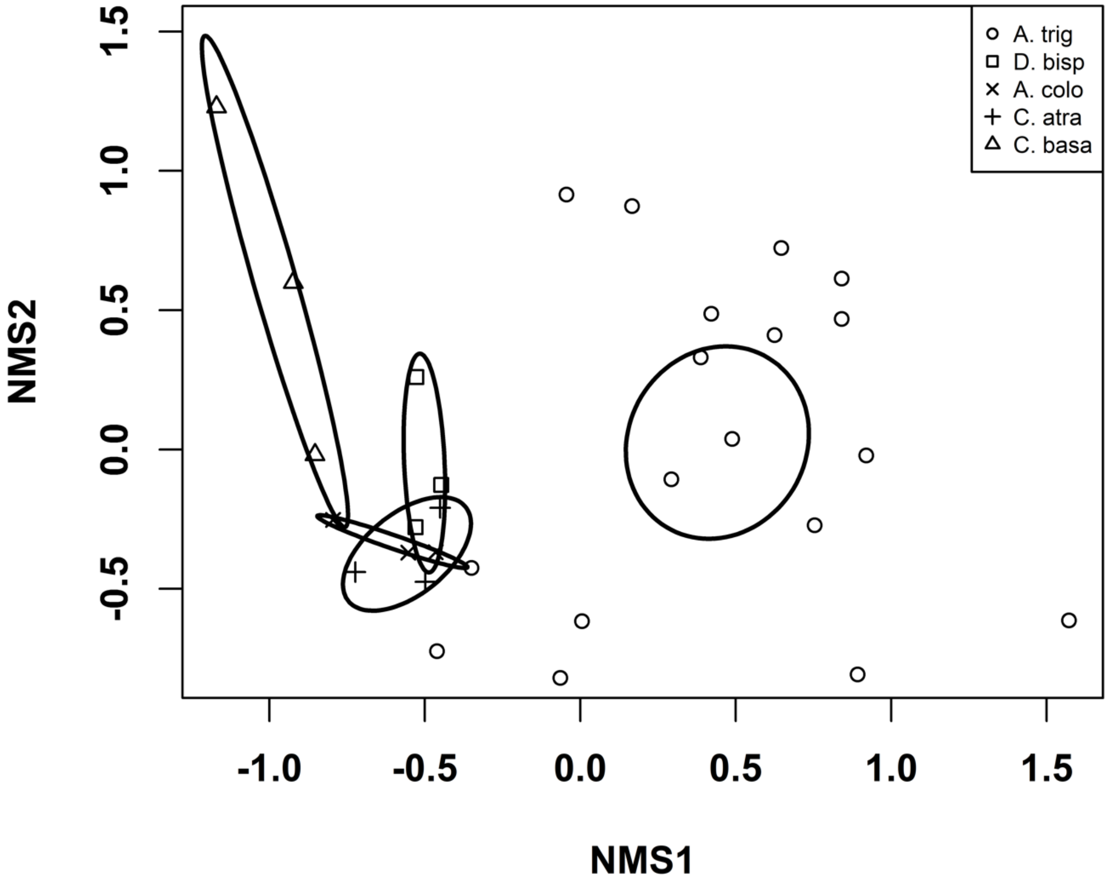
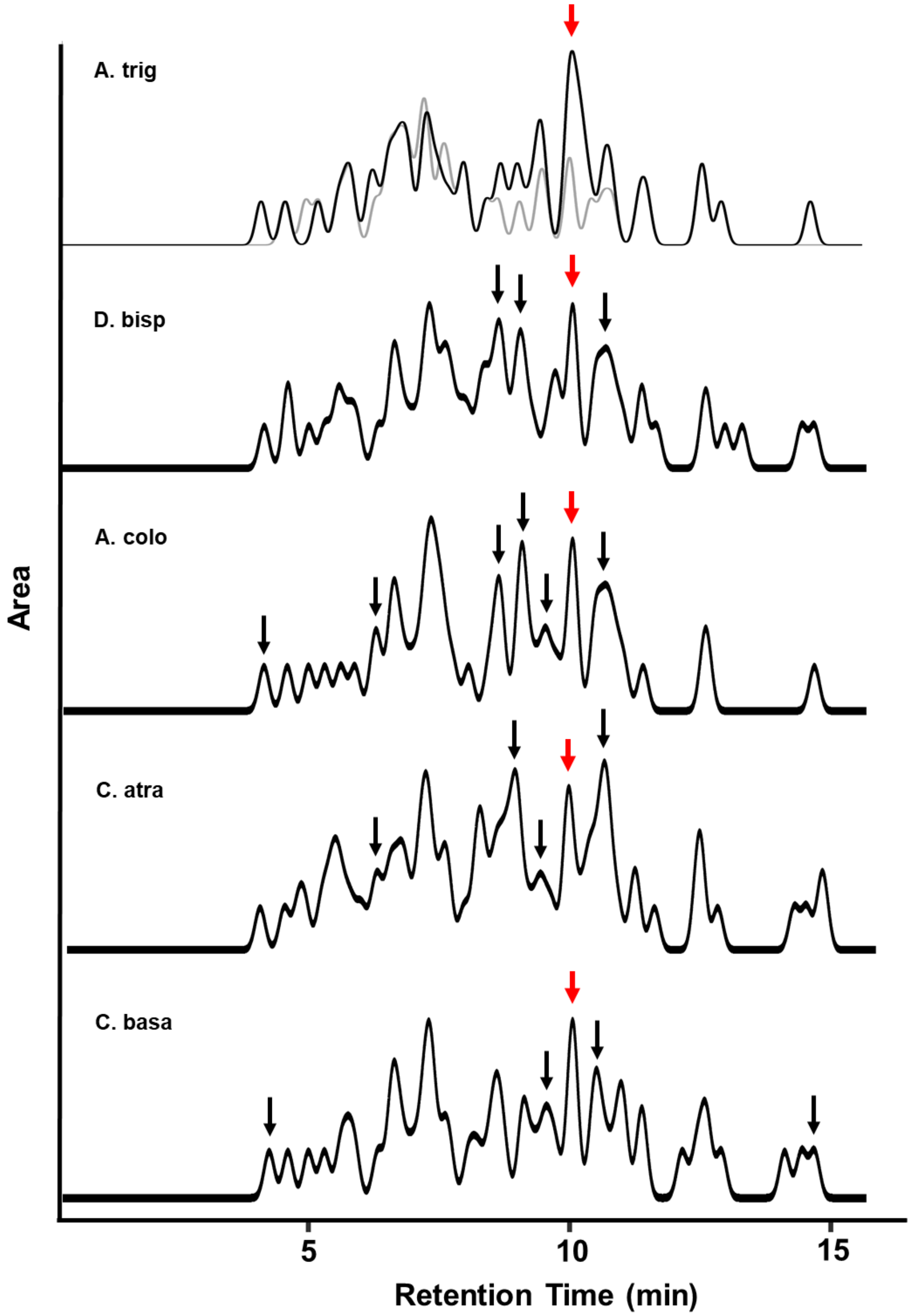


Figure 10.



A. trig
D. bisp
A. colo
C. atra
C. basa
NMS2
1.5
1.0
0.5
0.0
-0.5
-1.0
-0.5
0.0
0.5
1.0
1.5
NMS1

Figure 11.


A. trig
D. bisp
A. colo
C. atra
C. basa
Area
5
10
15
Retention Time (min)

CHAPTER III

NESTS OF A COMMON NEOTROPICAL ANT ARE CHEMICALLY SIMILAR TO THEIR LOCAL ENVIRONMENT

SUMMARY

Many species rely on chemical signals to communicate. Such signals can serve as reliable indicators of the presence of an organism when they persist in the local environment. The common Neotropical canopy ant, *Azteca trigona*, produces conspicuous alarm pheromones that are often detectable by humans over distances > 5 m. Here, I tested the hypothesis that *A. trigona* nests generate persistent odor plumes that distinguish them from the surrounding forest. I collected 360 open-air samples at 46 intact *A. trigona* nests and compared their chemical composition to similar samples of ambient forest air, worker alarm pheromones, and air near physically disturbed nests. The number of compounds detected among the different sources did not differ; however, *A. trigona* nest odor composition differed from that of worker alarm pheromones, and compounds associated with *A. trigona* alarm pheromones were more concentrated at disturbed nests. The chemical composition of *A. trigona* nests did not vary predictably with distance from the nest and the temperature, humidity, and time of day affected composition. Thus, I conclude that undisturbed *A. trigona* nests are chemically indistinguishable from the surrounding forest. Such potential chemical camouflage presumably is advantageous for the ants. Specifically, the potential cost of eavesdroppers is a strong selective pressure that may constrain the "leakage" of reliable, vulnerable signals.

INTRODUCTION

Many organisms produce chemical signals to convey reliable information to conspecifics and heterospecifics (Bradbury and Vehrencamp 2011). However, such signals often are emitted in the "public" environment, where they can be exploited by unintended receivers (i.e., eavesdroppers). Some chemical signals are maintained or persist in the environment for long periods of time, thus acting as extended phenotypes (Dawkins 1982, Schaedelin and Taborsky 2009, Bordereau and Pasteels 2011) that affect the behaviors and local distribution of eavesdropping species (Goodale et al. 2010).

Sites where large numbers of chemical communicators are located, such as at social insect nests, are particularly prone to discovery by eavesdropping (Hughes et al. 2008). Social insects are central-place foragers that rely on chemical signals, especially pheromones, to find resources and protect their colonies (Fadl Ali and Morgan 1990, Billen 2006). Pheromones benefit their emitters in many ways (e.g., maintaining mutualisms, facilitating access to resources, and reducing interactions with inter- and intraspecific competitors or predators; Hölldobler and Lumsden 1980, Hölldobler and Wilson 1990, Menzel et al. 2010, Adams et al. 2020). However, the same pheromones can be costly when exploited by eavesdropping predators, competitors, or parasites (Stowe et al. 1995).

Although eavesdropping on social insect pheromones is a well-known phenomenon, the mechanisms used by enemies and non-harmful associates to locate ant hosts remain unclear in most cases (reviewed by Adams et al. 2020). It is likely that pheromones near stationary ant nests (such as trail, defense, or nest-marking pheromones) are especially prone to eavesdropping as such pheromones are likely produced often and maintained over long periods of time (Slaa and Hughes 2009). In particular, large, exposed nest structures (e.g., carton nests) presumably have higher risk of detection than more cryptic nests in wood cavities or underground as pheromones produced near exposed nests are more likely to travel through the open air unimpeded (Adams et al. 2020). Carton nests also often harbor a suite of fungal and bacterial symbionts that release their own volatile compounds and may contribute to the suite of ant odors (Bahn et al. 2007, Voglmayr et al. 2011, Lucas et al. 2017). Thus, it is likely that a distinct chemical odor plume surrounds these nests, although this possibility remains untested.

Physics and fluid dynamics suggest that local environmental factors affect the characteristics of pheromones near ant nests. Specifically, odor plumes generally follow Gaussian dispersion dynamics (e.g., Bossert and Wilson 1963), and the concentrations of constituent compounds in air surrounding ant nests should decline predictably with distance from the source. However, even slight air currents modify plume dynamics, and different volatile compounds evaporate at different rates, which are also affected by temperature (i.e., faster under warmer conditions; Brown et al. 2011). Consequently, I expect that even in static air, the pattern of plume decay around large carton nests varies with their location due to changes in local abiotic conditions such as temperature and humidity.

The common Neotropical ant species-complex *Azteca chartifex/trigona* (Formicidae: Dolichoderinae; hereafter, *A. trigona* for simplicity) builds carton nests in trees that can persist in a single location (in full sun or under the dark forest canopy) for a decade or more (Wheeler 1986; Figure 2). Worker alarm pheromones are used to defend territorial boundaries (Adams 1994), and many co-occurring ant species exhibit distinct negative responses when exposed to *A. trigona* alarm pheromones (Wells et al., *in review*). *Azteca* carton nests are often covered in fungal and other microbial associates (Figure 12; Mayer and Voglmayr 2009, Lucas et al. 2017), and such nests are occasionally detectable by smell alone (RW *pers. obs.*), especially when recently disturbed. Moreover, some heterospecific ants will avoid *A. trigona* nest material (J. Manubay, *pers. comm*), although nest material does not match the cuticular hydrocarbon profiles of workers (Servigne et al. 2018). These observations suggest that the air space surrounding *A. trigona* nests has a distinct chemical composition that can be used both by colony members and by eavesdroppers far from nests. However, the chemical composition of these plumes and their existence in the absence of nest disturbance is unknown.

The goal of this study was to determine whether *Azteca trigona* nests emit a distinct, persistent, volatile chemical cue that could serve as a reliable indicator of their location. I used open-air chemical collection techniques to address whether: 1) the chemical composition of air immediately surrounding *A. trigona* nests (i.e., the odor plume) is distinct from that of the nearby forest; 2) the nest odor plume composition changes when the nest is disturbed; 3) the plume composition matches that of the volatile odors produced by *A. trigona* workers; 4) the plume composition changes predictably with distance from the nest; and 5) the plume composition changes predictably under different environmental conditions (e.g., temperature). I predicted that *A. trigona* nest odor composition would be distinct from the forest odor composition and that the full suite of compounds in nest odors would consistently include a subset of compounds that matches the composition of worker pheromones. Finally, given that volatile compounds evaporate faster under warmer conditions and *A. trigona* nests may be located in bright or dark areas of the forest (Brown et al. 2011), I predicted that samples collected when it was hotter and drier would have lower concentrations of compounds.

METHODS

This study was conducted on Barro Colorado Island (BCI) in Panama in the wet seasons between July 2016 - October 2018. BCI is a seasonally moist lowland forest (see Leigh et al. 2014 for further site descriptions) with an average *A. trigona* nest density of ca. 10 ha^{-1} (Wells et al., *unpublished data*).

Open-air Collections

I used an open-air pull technique to determine the chemical composition of air in close proximity to *A. trigona* nests. I collected a total of 360 open-air samples distributed among 46 different nests. All samples were collected between 08:30 and 16:30 during fair weather and calm conditions. I generally avoided using the same nest for more than one part of this study (with the exception of the Disturbance collections described below). In the few cases where a single nest was used to address different focal questions, the samples were taken in different years.

Up to four 4mm (outside diameter) glass tube chimneys, each containing a ~30mg PorapakQ filter, were supported on tripods at various locations around each nest (Figures 13-14). Filters were connected in pairs to a 6L min^{-1}, 12V diaphragm vacuum pump (Karlsson Robotics) via polypropylene tubing fitted with a T-terminus. Each sample in a given collection (see below) consisted of one filter through which air was drawn for one hour. Filters were wrapped in aluminum foil and transported to the University of Louisville for subsequent extraction and GC/MS analysis.

Question 1- Nest versus Forest: Do nest plumes differ from the surrounding forest air? To determine whether the chemical composition of the air in close proximity to *A. trigona* nests is distinct from the composition of the air in the surrounding forest, I collected open-air samples ca. 2 cm from the surface of 26 *A. trigona* nests. I similarly and simultaneously collected air samples at 26 paired control sites, which were haphazardly selected tree trunks in areas of forest that lacked conspicuous *A. trigona* nests (Figure 13). The paired control trees were at least 10 m away from the corresponding *A. trigona* nest.

Question 2 - Disturbance: Does physical disturbance create a distinct nest odor plume? Following the Nest vs. Forest collections described above, I disturbed 13 of the 26 nests by inserting a metal tripod one inch into the surface of the nest and scraping the surface longitudinally over a distance of ca. 25 cm. I then collected another set of air samples as described above, and repeated the scraping disturbance midway through the collection period.

Question 3 - Workers versus Nest: Does the composition of nest odors resemble pheromones produced by worker ants? I used the "headspace" collection technique (modified from Rodriguez-Saona et al. 2001) to determine if the chemical composition of air surrounding disturbed *A. trigona* workers (which presumably contains abundant alarm pheromones) differs from air surrounding undisturbed nests. After collecting open-air samples at an *A. trigona* nest (following the Nest vs. Forest methods described above), I placed five or more ants from the associated nest into each of three separate, clean 20 ml glass scintillation vials. Each vial was capped with aluminum foil through which the beveled end of a glass tube chimney filter was inserted (Figure 6). Three vials containing ants from the same nest, plus one empty vial (which served as an ambient air control), were connected in pairs to two vacuum pumps with polypropylene tubing per each nest (Figure 6). The pumps were operated for 30 minutes. Open-air samples and associated worker headspace samples were collected from six *A. trigona* nests.

Question 4 - Spatial Variation: Does plume composition change with distance from a nest? To determine if *Azteca* nests produce well-defined chemical plumes that decay with distance, I collected open-air samples at seven non-disturbed *A. trigona* nests. I collected sixteen open-air samples at each nest along four distances from the nest (four samples per distance at 0.02, 1, 2, and 5 m from the nest; Figure 14).

Question 5 - Environmental Variation: Does plume composition vary predictably with local abiotic conditions? I collected open-air odor samples at 30 additional *A. trigona* nests following the Nest vs. Forest collection methods described above (Figure 13). These samples were combined with the 0.02m nest samples from the Nest vs. Forest and Spatial Variation collections described above (Figure 14) to determine if the chemical composition of the air near nests varies with local environmental conditions. I measured ambient temperature (°C), relative humidity (%), and collection time at the beginning of each collection period.

GC/MS Analysis of Nest Odor Composition

I eluted each volatile collection filter with an internal standard that contained 150 µl dichloromethane with 10 ng μl^{-1} nonyl acetate (Rodriguez-Saona et al. 2001). Eluted samples were then analyzed with an Agilent 7890B Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA). The chromatograph was run in splitless mode with an inlet temperature of 250°C: the oven temperature was initiated at 35°C for sample injection and heated 15°C m^{-1} to 250°C with helium as a carrier gas at an average velocity of 22.5 cm s^{-1}. Samples were resolved on an Agilent DB-5 column (30 m length, 0.25 mm diameter, with a built-in 10 m DuraGuard pre-column). An Agilent 5977A Mass Spectrometer with an EI ion source detected analytes while in scanning mode (50-550 m/z with transfer line and ion source temperatures set at 230°C and 150°C). The MassHunter software suite (Agilent) determined peak area quantification after peak deconvolution and compounds were identified based on comparison to the NIST14 spectral library. I used the R package GCalignR to align cross-samples based on retention time (Ottensmann et al. 2018) and then double-checked alignments with base peak values. The concentration of each compound (ng min^{-1}) within a sample was estimated by calculating the area of each analyte against the internal standard. Final analytical concentrations for worker headspace samples were determined by subtracting analyte concentrations in the appropriate control sample from the corresponding ant worker samples.

Statistical analyses

All analyses were performed in the R statistical environment (R Core Team 2019) and in PRIMER (version 6.1.18). I used a community ecology approach to evaluate variation in the number and composition of compounds collected in our samples (i.e., treating compounds as species). For each focal question, I estimated the number of compounds likely to be present in the samples with compound (species) richness accumulation curves (*iNEXT* package; Chao et al. 2014; Hsieh et al. 2016; Figure 15).

I used four different generalized mixed-effect linear models using a Poisson error distribution for count data to determine whether the average number of compounds differed among the different collection locations (i.e., treatments; Table 13) in questions 1-4. Nested models were compared with likelihood ratio tests to determine significance between treatments. Treatment was the fixed effect and nest was the random effect (Table 13). For the Environmental Variation collections, I conducted a generalized linear mixed-effect model on count data, treating time of collection, temperature (°C), and relative humidity (%) as fixed effects and sample period and nest as random effects.

PERMANOVA and PERMDISP analyses were used to test for differences in chemical composition and beta diversity among treatments from our five collection types (Anderson et al. 2008). The PERMDISP analysis assesses the distance of samples from a treatment or grouping to the centroid of that treatment or grouping. Treatments with higher variability in compound composition between samples (i.e., higher beta diversity) will have a larger distance to the centroid. I created non-metric multidimensional ordinations (NMS) to visualize differences in odor composition among the treatments (Table 13) in these collections (*vegan* package). I calculated distance matrices using Bray-Curtis indices on square-root transformed concentration data, and used the resulting matrices as the basis for each PERMANOVA, PERMDISP, and NMS ordination. The Nest vs. Forest, Disturbance, Spatial Variation, and Worker vs. Nest models included treatments (Table 13) as the fixed effect and nest identity as the random effect. For the Environmental Variation model, temperature, humidity, and time of collection were treated as categorical fixed effects and sample period and nest were the random effects. I used indicator species analysis (*indicspecies* package; De Cáceres and Legendre 2009) to identify compounds that were strongly associated with significant terms from the PERMANOVA (Dufrêne and Legendre 1997).

Finally, I conducted species co-occurrence analyses (*EcoSimR* package; Gotelli and Ellison 2013) to determine if there were patterns of segregation and aggregation among compounds. Specifically, I used this model to determine if the detected compounds frequently overlapped among treatments, or were randomly distributed among treatments within a given focal question. I used the fixed-fixed model and generated 9999 random matrices for each analysis.

RESULTS

Question 1: Nest vs. Forest

The expectation that *Azteca* nests are surrounded by a distinct plume of volatile compounds was not supported. The air within a few centimeters of *Azteca* nests was chemically indistinguishable from the surrounding forest air. The number of compounds detected in open-air samples did not differ between ant nests and nearby control trees (GLM likelihood ratio test $\chi^2 = 0.0038$, df = 1, P = 0.95). Nor did the chemical composition (*Pseudo*-$F_{1,51} = 0.72$, P = 0.67; Figure 16) or beta diversity ($F_{1,50} = 0.56$, P = 0.54) differ between the treatments. Moreover, nest and control treatments shared eight of the ten most abundant compounds (Table 14), and there was a low frequency of compound co-occurrence (or segregation) in samples from each treatment (observed ≥ simulated, P = 0.001 for controls and nests).

Question 2: Disturbance

The expectation that disturbed nests would produce a distinct odor plume was only weakly supported. Specifically, disturbance caused a spike in the concentrations of 2-Heptanone, a component of *Azteca* alarm pheromones (Amoore et al. 1969) and decahydro-4a-methyl-1-Napthalenol, a naphthol that has not been previously found in insects (Table 14). Additionally, the compound Iridomyrmecin was detected only in samples from disturbed nests. This compound previously was known only from *Iridomyrmex* spp. ants (Dolichoderinae; Blum et al. 1966, Attygalle and Morgan 1984).

Although the ten most concentrated compounds detected among nest sites were variable, the number of compounds did not differ among disturbed nests, undisturbed nests, and control sites ($\chi^2 = 0.051$, df = 1, P = 0.82). Likewise, open air samples from disturbed nests were similar in chemical composition (*Pseudo*-$F_{2,38} = 1.71$, P = 0.090; Figure 17) and beta diversity ($F_{2,36} = 0.64$, P = 0.60) to undisturbed nests and control sites. Whereas the most concentrated compound detected at disturbed nests was 2-Heptanone, the plant volatile 1-(4-ethylphenyl)- Ethanone was the most concentrated compound detected at the undisturbed nests (Table 14). Although there were clear differences in the concentrations of some compounds between the two nest sites, there was a low frequency of compound co-occurrence among disturbed and undisturbed nests (observed ≥ simulated, P = 0.001 in all cases), and these nest sites shared seven of the ten most abundant compounds (Table 14).

Question 3: Worker vs. Nest

The average number of compounds detected in *A. trigona* worker headspace samples was slightly lower than the number of compounds in the air space surrounding *A. trigona* nests ($\chi^2 = 3.84$, df = 1, P = 0.0501; Table 13). The composition of the headspace samples also differed from that of the nest samples (*Pseudo*-$F_{1,11} = 3.32$, $P = 0.026$; Figure 18). Additionally, there was no difference in the amount of turnover between the worker and nest samples (i.e., there was no difference between the distances to the centroid between the treatments; $F_{1,10} = 0.19$, $P = 0.75$). 2-Heptanone was the only indicator compound significantly associated with worker headspace samples (IndVal = 0.98, $P = 0.003$), and was 21 times more concentrated (on average) in the worker vs. nest samples (Table 14). 3-Carene, a plant metabolite, was the only indicator compound associated with the open-air samples (IndVal = 0.91, $P = 0.027$; Table 14).

Question 4: Spatial Variation

The number of compounds detected at nests did not decrease with distance from the nest ($\chi^2 = 2.46$, df = 3, $P = 0.48$). Distance also had no effect on the composition (*Pseudo*-$F_{3,27} = 1.06$, $P = 0.4$; Figure 19) or beta diversity ($F_{3,24} = 1.92$, $P = 0.29$) of compounds surrounding the nests. The detected compounds had high co-occurrence frequencies at 0.02m (observed $\geq$ simulated, $P = 0.001$), 2m (observed $\geq$ simulated, $P = 0.027$), and 5m (observed $\geq$ simulated, $P = 0.006$). However, the chemical compounds collected in the 1m samples and among the seven nest sites were neither segregated nor aggregated (1m: observed $\leq$ simulated, $P = 0.64$; observed $\geq$ simulated, $P = 0.39$; Site: observed $\leq$ simulated, $P = 0.71$; observed $\geq$ simulated, $P = 0.30$). The majority of the ten most abundant compounds at each distance were plant-associated compounds, whereas 2-Heptanone was one of the most abundant compounds at 1 meter (Table 14).

Question 5: Environmental Variation

The chemical compounds detected around *A. trigona* nests were influenced by environmental factors. There was a significant interaction among temperature, time, and humidity on the average number of nest compounds detected at each nest ($\chi^2 = 5.67$, df = 1, P = 0.017).

DISCUSSION

Animals that produce volatile pheromones or similar chemical signals are vulnerable to eavesdropping in part because the signal cannot be controlled once emitted. Here I show that the odorous and aggressive Neotropical ant species, *Azteca trigona*, maintains nests that apparently are chemically indistinct from the local environment. The diversity and composition of chemicals in the air near nests resemble those of ambient forest air under similar environmental conditions. Contrary to our expectations, *A. trigona* nests do not continuously emanate detectable odors that chemically resemble volatile pheromones produced by the workers.

The results of this study provide at least some support for the expectation that disturbed nests produce chemically distinct odor plumes. Specifically, 2-Heptanone (a compound that is widespread in social insect alarm pheromones; Blum and Brand 1972), 6-methyl-5-hepten-2-one (sulcatone), and other likely ant-related compounds (such as Iridomyrmecin) had higher concentrations in air surrounding disturbed nests; however, disturbance of nests did not create a compositionally distinct odor plume. Nonetheless, the increased concentration of ant-related compounds at disturbed nests suggests that an *A. trigona* nest could reliably be distinguished or located by eavesdroppers when workers around the nest become disturbed.

The absence of a distinct odor plume surrounding undisturbed *Azteca* nests presumably protects colonies from eavesdroppers and other threats. For example, *Azteca* nest densities are affected by top-down predators and parasitoids such as phorid flies (Vandermeer et al. 2008). Phorid flies detect *Azteca* workers by eavesdropping on their alarm pheromones (Mathis et al. 2011), and phorids commonly attacked workers at nests that were disturbed in this study (RW, *pers. obs.*). Anteaters (specifically *Tamandua*) also presumably pose a threat to *Azteca* colonies (Hirsch et al. 2014), but the relevance of nest odor to this interaction is unknown. Although physical damage to a nest presumably increases the risk of attack by eavesdroppers, damaged *A. trigona* nests are very uncommon on BCI (RW, *pers. obs.*).

The prediction that worker headspace chemical composition would be a subset of nest odor composition was not supported, suggesting that workers do not contribute volatile odors to undisturbed nest material. Although I did not test for the presence of worker cuticular hydrocarbons (CHCs) on the nest material, CHCs are used to mark the entrances to some social insect nests (Butler et al. 1969, Lenoir et al. 2009). However, *A. chartifex* carton nest CHC composition in French Guiana is distinct from worker CHCs and similar to wasp nest CHCs and other plant material (Servigne et al. 2018), further supporting the conclusion that nest odors are chemically indistinct from their local environments.

The volatile chemical contributions of fungal and microbial associates on the external surfaces of *A. trigona* carton nests are unknown and deserve further study. Compounds produced by these associates presumably contribute to the lack of a distinct chemical composition surrounding *A. trigona* nests and should vary based on interspecific microbial interactions (Weisskopf et al. 2021). Given the large number of plant-associated compounds that occurred in our samples, it is possible that some chemical compounds produced around the nest or by *A. trigona* ants were undetected or masked by other common volatiles. Many insects can detect compounds at very low concentrations, and such compounds likely were undersampled by the methods used in this study. I pose a suite of questions for future research that would at least partially address these concerns: 1) Which, if any, compounds surrounding *A. trigona* nests are detectable by workers, and at what concentrations are they detectable?; 2) How often are *A. trigona* nests disturbed, and how does disturbance affect their exposure to eavesdroppers?; and 3) Do external nest associates have a chemically-mediated mutualistic relationship with *Azteca* (i.e., contributing to the chemical camouflage of the nests while receiving substrate and protection from the ants)? Answering these questions was beyond the scope of this study, but I believe they provide potentially fruitful avenues for future research.

Azteca ants are a common genus of canopy ants that rely on chemical signals to coordinate nest activities. *Azteca* ant workers are aggressive and produce pungent odors that are used by some people as biological control agents and insect repellent (Overal and Posey 1984, Posey 1991). Here I show that the carton nests of *A. trigona* do not have a unique odor in the environment. Instead, these nests seem to hide the pungent odors of the workers they contain, allowing the nests to be chemically silent in their environment. This chemical silence could be an adaptive mechanism to avoid detection, or could be an indirect consequence of using dead (and less odoriferous) plant material for nest construction. Regardless, the lack of chemical detection (i.e., being chemically cryptic) could protect nests and nest resources from potential predators and parasitoids, ultimately contributing to the ecological and evolutionary success of *A. trigona* and potentially other ant species.

TABLES

Table 13. Summary of sampling effort for each major component of this study. *Nests* = the number of different *Azteca* nests sampled; *Samples* = the number of exposed filters per treatment used in GC/MS analysis; *Compounds* = the average (± SE) number of compounds detected per treatment; and *Sampling Completeness* = the percent (%) of compounds actually detected relative to the total number of compounds that are likely present in each treatment based on rarefaction analyses (Figure 16).

Question	**Nests**	**Treatments**	**Samples**	**Compounds**	**Sampling Completeness (%)**
1) NEST VS. FOREST	26	Forest	104	45.2 ± 1.97	100
		Nest	104	45.3 ± 1.87	100
2) DISTURBANCE	13	Disturbance	52	48.7 ± 2.98	100
		Nest	52	48.1 ± 3.23	100
3) WORKER VS. NEST	6	Nest	24	38.2 ± 5.56	97
		Workers	24	31.3 ± 8.64	98
4) SPATIAL VARIATION	7	0.02m	28	20.9 ± 2.55	100
		1m	28	19.1 ± 1.40	97
		2m	28	17.4 ± 1.81	99
		5m	28	20.6 ± 1.39	99
5) ENVIRONMENTAL VARITATION	30	NA	120	40.9 ± 1.40	100

Table 14. The most abundant compounds detected from each major component of the study and their corresponding treatments. Values under *Headspace* and *Nest* are average concentrations (ng min^{-1}). *Compound Name* = NIST14 library match based on NIST14 spectral match confidence of > 80%. * = compounds associated with a given treatment based on indicator species analysis. Compounds are arranged from the highest to lowest average concentration associated with any treatment.

Compound Name	**NEST VS. FOREST**		**DISTURBANCE VS NEST**		**WORKER VS NEST**		**SPATIAL VARIATION**				**ENVI. VARI-ATION**
	Control	Nest	Disturbed	Nest	Work-ers	Nest	0.02m	1m	2m	5m	
2-Heptanone			12		64*	3		0.4			2
Oleic Acid						19					
di(2-propylpentyl) ester Phthalic acid	3		6	4		7	17	1	3	8	5
decahydro-4a-methyl-1-Naphthalenol			16		3						
Diethyltoluamide					13	7	1	1	1	1	4
6-methyl-5-Hepten-2-one					13						
2,2,4,4,6,8,8-heptamethyl-Nonane			11								
p-Cymen-7-ol	7	6	8	8							9
1-(4-ethylphenyl)-Ethanone	7	7	8	8	4						6
1-methyl-Cyclopentanol		5		8				1	1	0.5	3
Toluene	3	3					2	2	2	2	8
Methylene chloride	3	4		5			7	7	6	6	3
2-Ethyl-1-hexanol	4	4	5	6			5			0.4	

Limonene	3	2				5					
UNKNOWN 2					5						
1,4-diethyl-Benzene	3	3	4	4							
Mesitylene	3	3	3	4							
(1-methylethyl)-Benzene		3	3	4							
3-Carene						4*		0.3			
4-oxo-Pentanoic acid						4					
1,3-dimethyl-Benzene					2	3					
Naphthalene				3			1			0.2	2
.beta.-Phellandrene	3										
1,3-diethyl-Benzene											3
4-methyl-1-(1-methylethyl)-Bicyclo[3.1.0]hex-2-ene						3					
9-Nonadecene					3						
Vanillin					3						
.alpha.-methyl-acetate Benzenepropanol											2
Benzoic Acid						2					
Homosalate					2						
2-ethylhexyl ester Benzoic acid							1	0.2	0.2	0.2	
UNKNOWN 1							1				
Nonanal							0.5	0.3	0.3	0.3	

Tetradecane	0.5			
2-methyl-Naphthalene		0.2	0.3	0.3
Caprolactam			0.2	

FIGURE LEGENDS

Figure 12. The free-hanging carton nest of an *Azteca trigona* colony. This nest is ca. 2.5 meters long.

Figure 13. a) Diagram of the open-air sampling method. Tripods supported pairs of filters connected to a T-terminus ca. 0.02m from the surface of an *Azteca trigona* nest. Two pumps were connected to one battery for sampling. b) Open-air sampling apparatus in place at a control tree (i.e., lacking *Azteca trigona*). c) Open-air sampling apparatus used to collect volatile compounds within a few cm of the surface of an *Azteca trigona* nest.

Figure 14. Open-air sampling setup for the Spatial Variation question. Tripods supported collection filters at a consistent height above the ground at four different distances along two transects extending approximately opposite directions from an *Azteca trigona* nest (0.02, 1, 2, and 5m).

Figure 15. Compound richness accumulation curves for the five different questions. Data are from: a) Nest vs. Forest 104 open-air samples taken at 26 non-disturbed *Azteca* nests (Nest) and nearby forest sites (Control) lacking *Azteca* nests; b) Disturbance 52 open-air samples taken at 13 non-disturbed (Undisturbed) and disturbed (Disturbance) *Azteca trigona* nests; c) Spatial Variation 64 open-air samples collected at 0.02, 1, 2, and 5 meters from *Azteca trigona* nests; d) Worker vs. Nest 24 open-air samples and 18 corresponding worker headspace samples collected from *Azteca trigona* nests; and e) Environmental Variation 120 open-air samples collected from 30 *Azteca trigona* nests on Barro Colorado Island. Shading

shows 95% confidence intervals. Dashed lines indicate the estimated compound richness for each treatment.

Figure 16. Non-metric multidimensional scaling ordination of the chemical concentrations for 26 non-disturbed *Azteca trigona* nests (filled circles) and corresponding forest sites lacking *A. trigona* nests (Forest; open circles). Ellipses represent 95% confidence intervals from the centroid of each group (solid line = *Azteca* nests; dashed line = control sites). Stress = 0.094.

Figure 17. Non-metric multidimensional scaling ordination of the chemical concentrations for 13 non-disturbed *Azteca trigona* nests (Undisturbed; filled circles) that were then disturbed (Disturbance; open squares). Ellipses represent 95% confidence intervals from the centroid of each group (solid line = *Azteca* nests; dashed line = control sites). Stress = 0.092.

Figure 18. Non-metric multidimensional scaling ordination of the chemical concentrations of six *Azteca trigona* nests (closed circles) and worker volatile odors (Headspace; open triangles). Ellipses show 95% confidence intervals from the centroid of each group (solid line = *Azteca* nests; dotted line = worker odors). Stress = 0.12.

Figure 19. Non-metric multidimensional scaling ordination of the chemical concentrations compared between four distances (0.02m, 1m, 2m, and 5m) at seven non-disturbed *Azteca trigona* nests. Ellipses represent 95% confidence intervals from the centroid of each group (solid line = 0.02m; dotted line = 1m; dashed line = 2m; dash-dotted line = 5m). Stress = 0.13.

FIGURES

Figure 12.

Figure 13.

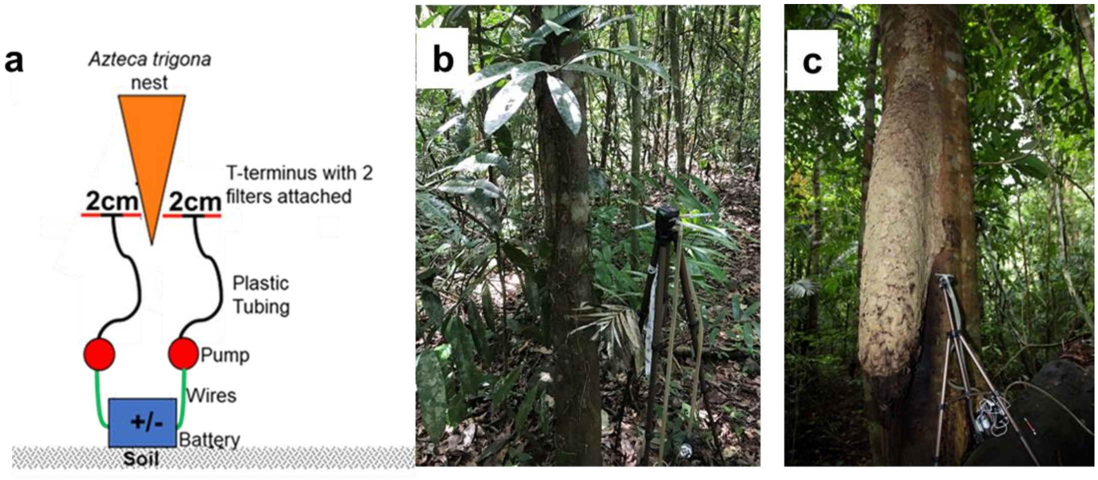


Figure 14.

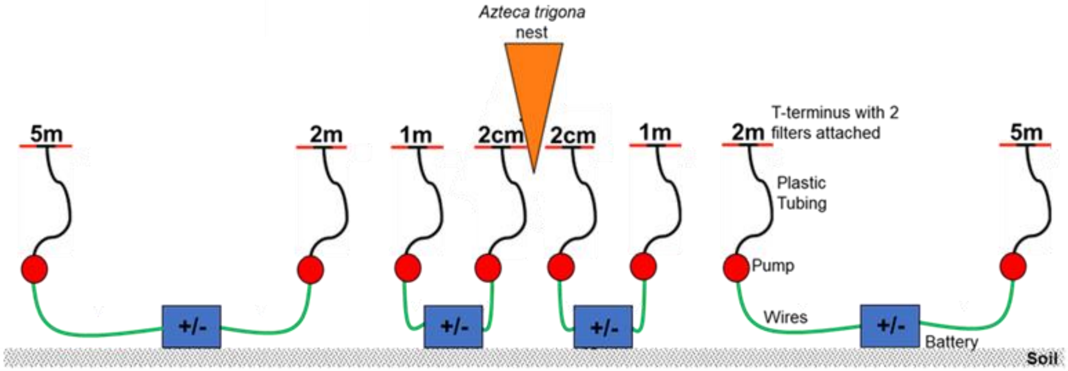


Figure 15.

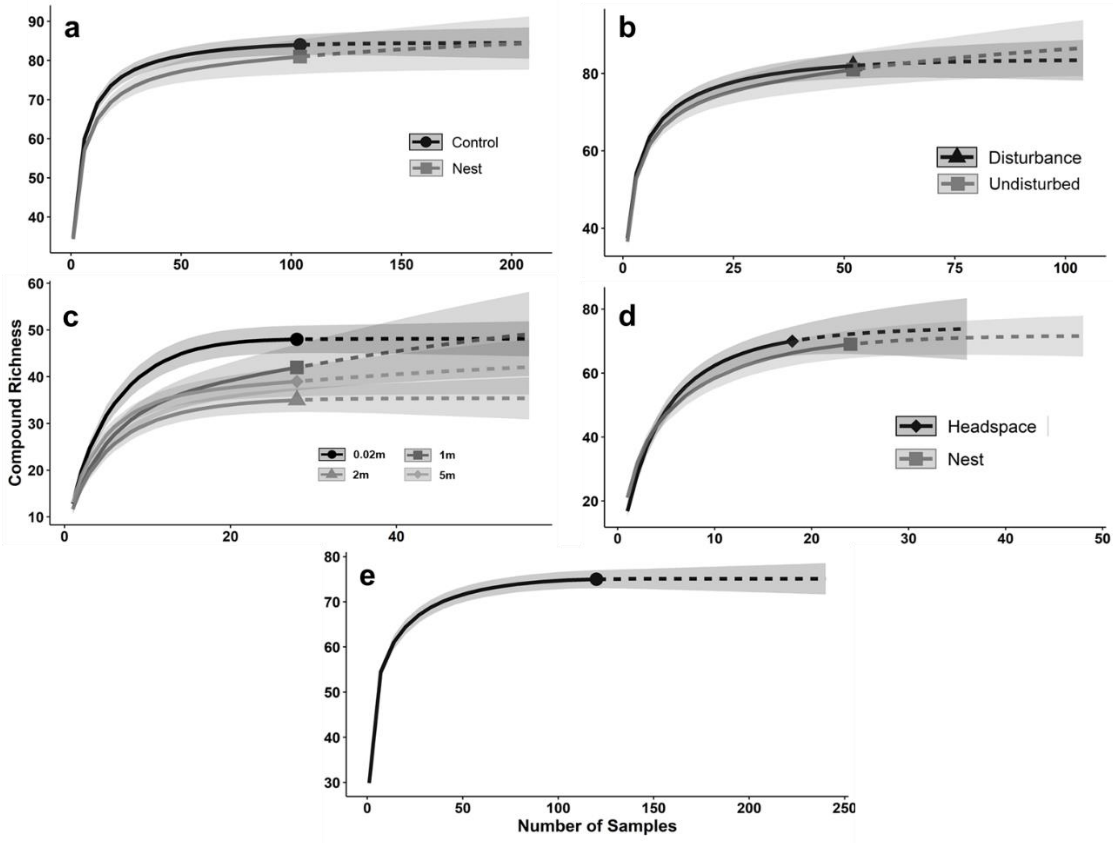

a
90
80
70
60
50
40
0
50
100
150
200
Control
Nest
b
80
60
40
0
25
50
75
100
Disturbance
Undisturbed
c
Compound Richness
60
50
40
30
20
10
0
20
40
0.02m
1m
2m
5m
d
80
60
40
20
10
20
30
40
50
Headspace
Nest
e
80
70
60
50
40
30
0
50
100
150
200
250
Number of Samples

Figure 16.

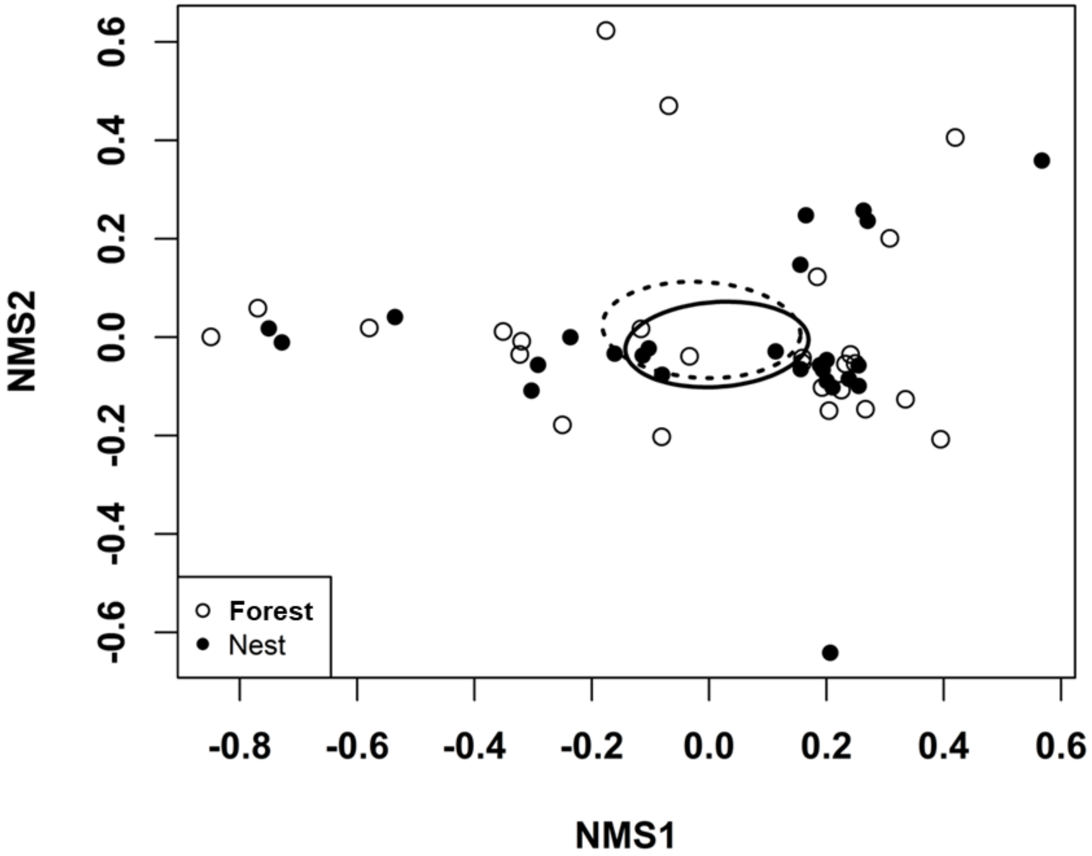
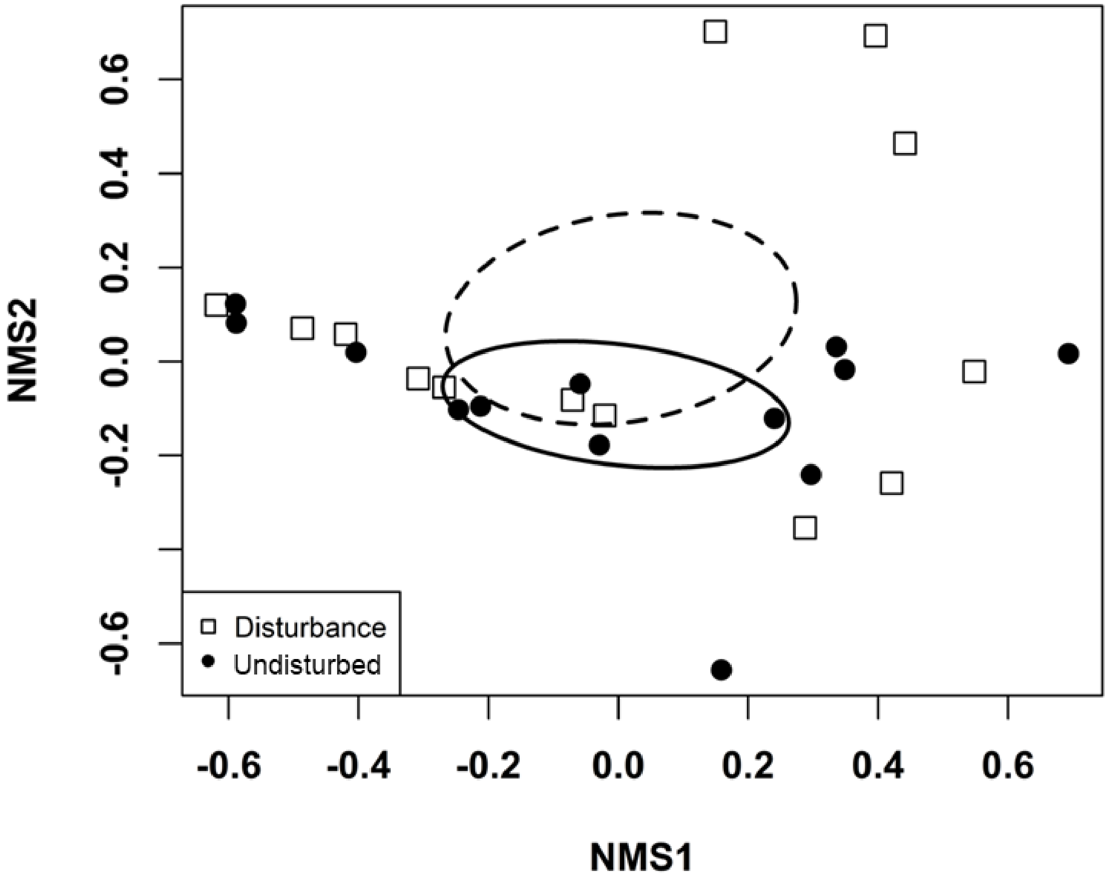


Figure 17.



NMS2
0.6
0.4
0.2
0.0
-0.2
-0.6
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
NMS1
□ Disturbance
● Undisturbed

Figure 18.

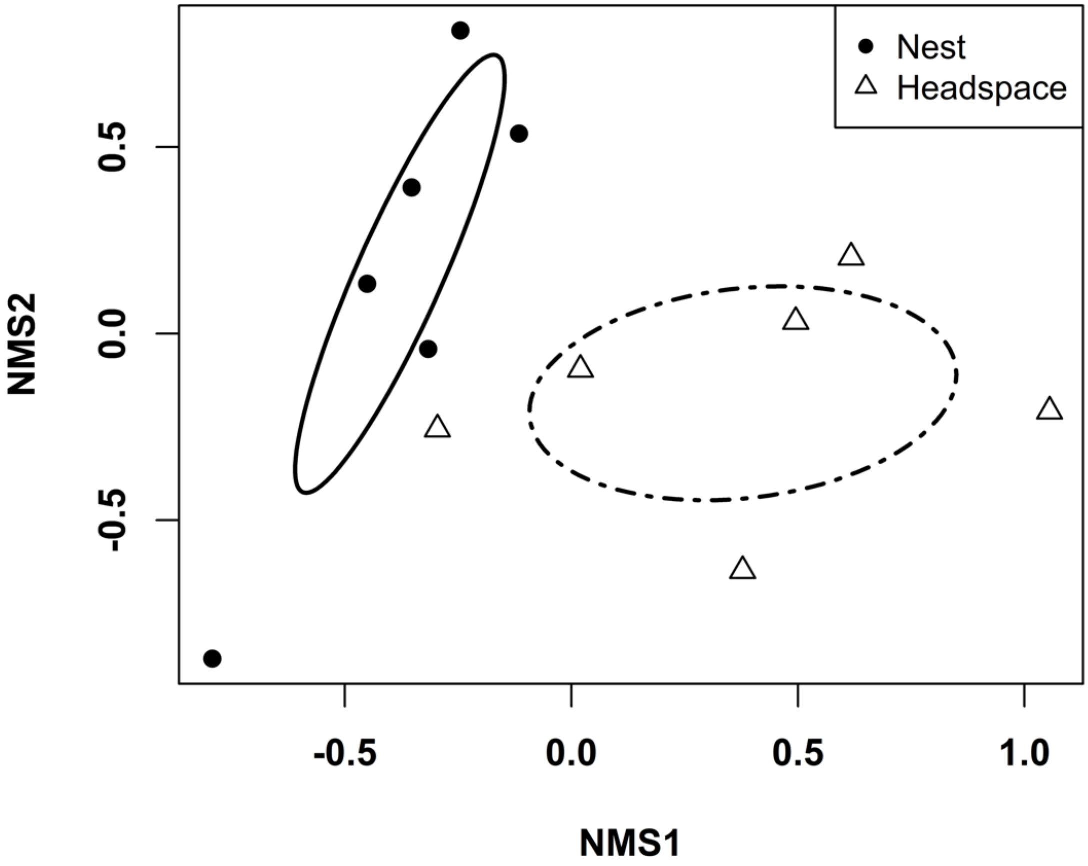
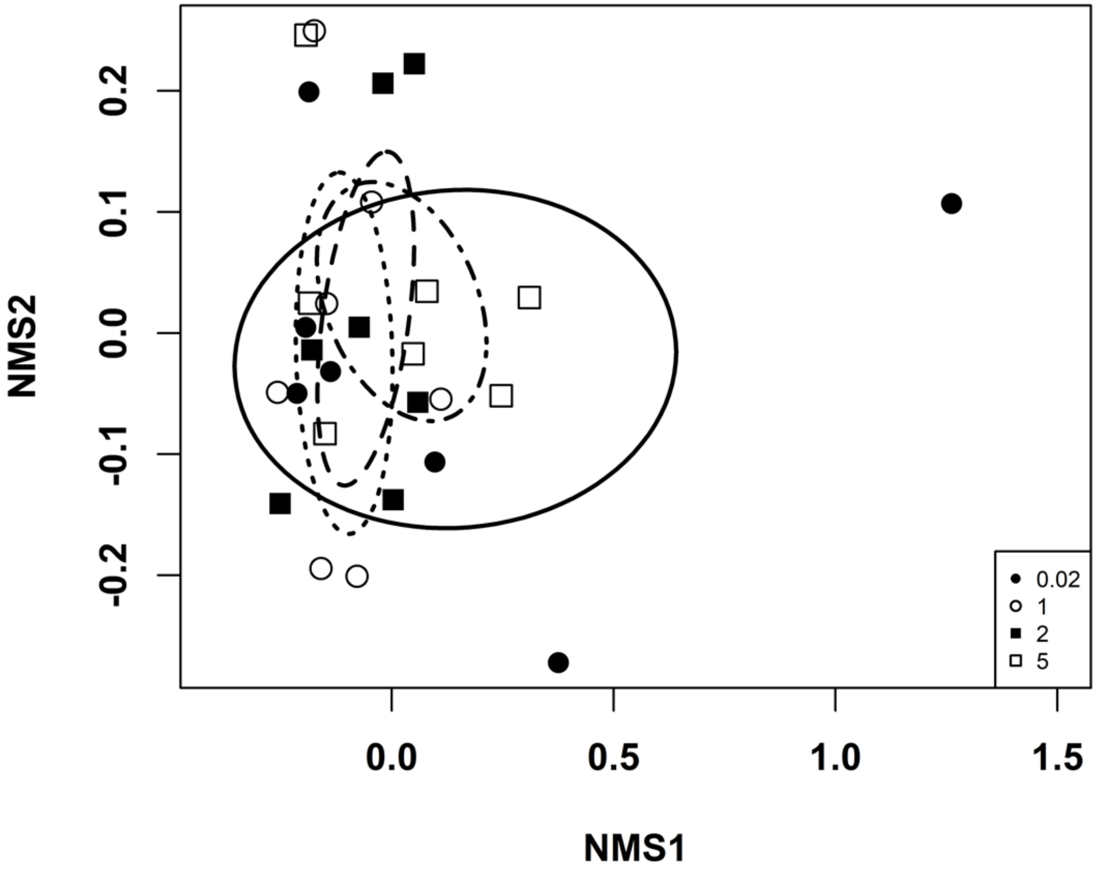


Figure 19.



CHAPTER IV

EXPLORING COMPETITION AMONG CANOPY ANTS: A DOMINANT ANT AFFECTS ACCESS TO NEST RESOURCES, BUT ONLY AT SMALL SPATIAL SCALES

SUMMARY

Competition is an important agent in community assembly and shapes local community structure. In the tropical rainforest canopy, behaviorally dominant ants commonly exhibit interference competition for artificial food and nest resources; however, the consequences of such interactions for arboreal ant community structure remain unresolved. Here, I examine the effects of an ecologically dominant canopy ant species, *Azteca chartifex/trigona*, on the composition of resident ants and of ants colonizing artificial nest resources in a lowland forest of Panama. I added four types of artificial nest substrates to 19 *Dipteryx oleifera* trees, 10 with resident *A. trigona* colonies and 9 lacking *A. trigona*. I measured the frequency of occupation and species composition of ants in the artificial nests after one year. I also conducted ant surveys in 28 tree crowns to determine how *A. trigona* presence influences community structure at the individual tree level. The presence of *A. trigona* affected the composition of ants colonizing the artificial nests, slightly affected the frequency of nest occupancy, but did not affect the species richness or composition of ants in a tree. Specifically, *Dolichoderus bispinosus* was associated with artificial nests in trees without *A. trigona*, whereas *D. laminatus* was associated with nests in *A. trigona* trees. Moreover, the frequency of artificial nest occupancy in *A. trigona* trees was greater in areas of the tree where *A. trigona* were foraging. Collectively, these results indicate that *A. trigona* can interfere with

patterns of tree colonization by ants, but overall effects of *A. trigona* on arboreal ant community structure are relatively subtle in this forest.

INTRODUCTION

The availability of resources in the environment is a key factor shaping species interactions and local community structure (Tilman 1982, Rosenzweig 1995). Competition for food or mates is a key factor in community assembly processes (Stewart and Levin 1973, Tilman 1982). In some cases, species that are locally very abundant and aggressive can interfere with the ability of other species to access essential resources (i.e., interference competition; Whittaker 1965, Morse 1974, Dayton 1975). Such ecologically dominant species (i.e., those that are both relatively abundant and aggressive; hereafter, dominant species) can further exacerbate availability of resources by directly or indirectly outcompeting interspecific species for them (Morse 1974, Pimm et al. 1985).

Competition is considered the key mechanism that structures ant communities, especially within tropical rainforest canopies (Hölldobler and Wilson 1990, Majer et al. 1994, Lach 2007). Ants account for up to 90% of the canopy arthropod biomass, and canopy ants in particular represent up to a third of total ant species richness in lowland tropical forests (Tobin 1995, Davidson and Patrell-Kim 1996, Longino and Colwell 2020). Canopy ant biomass is often dominated by one or two aggressive species. These ecologically dominant species commonly exhibit aggressive exclusion behaviors (i.e., interference competition) at resource patches. This phenomenon is best documented at highly enriched food resources like tuna and honey baits (Adams 1994; Blüthgen and Stork 2007; Ribeiro et al. 2013).

In some settings, competitive interactions among canopy ants result in patterns of species exclusion and aggregations, known as an ant mosaic (Room 1971, Leston 1978, Majer and Delabie 1993, Mottl et al. 2021). However, empirical support for the ant mosaic hypothesis is mainly limited to relatively simple systems like agroecosystems or secondary forests; evidence that ecologically dominant ants shape community structure in more structurally complex forests is lacking (Ribas and Schoereder 2002; Sanders et al. 2007; but see Ribeiro et al. 2013). Evaluating if and how ecologically dominant species affect interspecific access to resources in more structurally complex forest systems should provide a better understanding of community structure in such systems (Achury et al. 2020).

Competition for nest sites likely plays a large role in structuring arboreal ant communities (Philpott and Foster 2005, Mottl et al. 2020). Niche differentiation among multiple types of nesting substrates should increase the number of species that co-occur within the same tree crown (Camarota et al. 2020). Arboreal ants occupy a variety of nesting substrates including canopy soils, tree bark, and hollow twigs or other wood cavities (Longino 2003, 2009, Mackay and Mackay 2010). Additionally, some ant species occupy more than one type of nesting substrate, such as soil and hollow twigs. Subdominant ants will shift nest-site usage to non-preferred cavity nests in the presence of dominants (Camarota et al. 2020). Cavity nesting species vary in their preferences for cavity entrance size, volume, and material (Philpott and Foster 2005, Jiménez-Soto and Philpott 2015, López-Dávila et al. 2021) ; however, the extent of nest site specificity is unknown for most ant species (De Medeiros et al. 1995; Blüthgen et al. 2000; Adams et al. 2017; but see Camarota et al. 2020). Given that the majority of ants in a tree crown are not dominant species, understanding the distribution of ants among nest resources will improve our understanding of patterns of local diversity.

The arboreal ant species complex, *Azteca chartifex/trigona* (Dolichoderinae; hereafter, *A. trigona*), is a widespread and ecologically dominant Neotropical ant (Wheeler 1986, Longino 2007). Workers of *A. trigona* aggressively defend foraging territories and can exclude other species in an ant mosaic pattern, at least within mangrove forests or at ant baits (Adams 1994, Ribeiro et al. 2013). This aggressive behavior also likely affects nest occupancy; a related study showed that *A. trigona* was a common occupant of artificial nests, and apparently prevented subdominant ants from colonizing those nests (Adams et al. 2019). *Azteca* also exclude heterospecific ants from colonizing artificial twig nests on coffee farms in Mexico (Philpott 2010). Presumably, the exclusion of cavity resources by *Azteca* could contribute to local species extinctions in a tree crown or cause co-occurring species to shift occupancy to other available nest types (Camarota et al. 2020). However, the effects of *Azteca* on nest occupancy, and the consequences of nest site competition for local species richness and composition, are not well understood in mature tropical forests.

The goals of this study were to determine if ant colonization of artificial nests is affected by the presence of *A. trigona* and if the presence of *A. trigona* shapes local ant community structure in a lowland tropical forest. I asked: 1) is there a negative association between the presence of *A. trigona* ants and occupancy of artificial nests by heterospecific ants, and 2) does *A. trigona* presence alter ant species richness and composition within a tree crown? I predicted that artificial nests in trees with *A. trigona* colonies would have lower occupancy, especially in artificial nests within the territorial boundaries of *A. trigona* colonies. Because *A. trigona* are ecologically dominant, I predicted that the species composition of occupied nests would differ between trees with and without *A. trigona*. Specifically, I expected lower species richness and more similar species composition among artificial nests in trees with *A. trigona* than in tress without *A. trigona*. Given the diversity of nesting habits among canopy ant species, I also predicted that species composition in occupied soil nests would differ from cavity nests, but frequency of occupants would be similar. Lastly, I predicted the arboreal ant species richness would be lower and species composition would be more similar among trees with *A. trigona* colonies versus trees without

A. *trigona*. I tested these predictions among 28 *Dipteryx oleifera* trees in a lowland forest of Panama.

METHODS

Study Site

I conducted fieldwork during the wet season (May-December) of 2018 and 2019 on Barro Colorado Island (BCI) in Panama (09°09'15" N, 79°50'50" W). BCI is a tropical moist forest with average annual rainfall of 2600 mm (Croat 1978, Windsor 1990). The arboreal ants on BCI are relatively well-documented, making this an ideal location for studies on canopy ant community structure (Montgomery 1985, Adams et al. 2017). Additionally, *A. trigona* colonies are common on BCI, with an average nest density of ca. 10 ha^{-1} (Wells et al., *unpublished data*, see chapter 5). Further site descriptions can be found in Croat (1978) and Leigh et al. (2014).

Ant surveys were conducted only in *D. oleifera* trees, an emergent canopy tree on BCI. I focused on a single tree species to avoid potentially confounding effects of tree species identity with arboreal ant community structure (Ribas et al. 2003, Adams et al. 2017). I chose *D. oleifera* trees because they have high ant species richness relative to other common canopy trees on BCI (Yanoviak and Kaspari 2000, Adams et al. 2019) and their dense wood makes them safe to climb. Focal tree crowns were accessed for all artificial nest additions and ant surveys using the single rope climbing technique (Perry 1978; Figure 20). All of the focal *D. oleifera* trees contained lianas, which potentially provide important resources for canopy ants (Blüthgen et al. 2000, Schnitzer et al. 2012, Adams et al. 2017, 2019).

Artificial Nest Additions

I created four types of artificial nests for this study: bark, cavity, soil, and twig nests (Figure 21). I used modified Dendy Larval Samplers (BioQuip Products, Inc. USA) to simulate the bark crevices and cavities that are commonly colonized by ants. Three of the four sides of the bark nests were covered with hardboard pieces, and I painted the exterior surfaces with latex paint to increase nest longevity under field conditions (Figure 21c). Each nest was tied to the tree so that the open side touched the tree bark (Figure 22a). I constructed cavity nests from blocks of native hard wood (5 x 5 x 15 cm; *Anacardium excelsum*) by drilling cavities at the base of the wood and creating a smaller entrance hole connecting to the middle of the cavity. To ensure the small entrance hole was the only entry into the cavity nests, I covered the cavity hole with a mixture of glue and sawdust. A hole in the top of the nest was used to attach the nest to the tree with rope (Figure 21d). I constructed soil nests from plastic pots filled to 75% capacity with organic compost soil (Aboquete Products, Boquete, Panama; Figure 21b). Twig nests were constructed by drilling a hole through the center of wooden dowel rods (Figure 21a) and tying ropes around the middle for tree attachment (Figure 22c).

I added five sets of artificial nests in each of 19 (10 with an *A. trigona* colony and 9 without *A. trigona*; unbalanced design due to time constraints) mid-sized *D. oleifera* trees (50-100 cm diameter at breast height) during the wet season of 2018 (Figure 22). Each set contained one each of bark, cavity, soil, and twig nests. Nests within a set were separated from each other by about 1 m and each set of nests was separated from the other four sets by ≥ 3 m. I secured the artificial nests with ropes to branches or lianas near the central axis of the tree. In the *Azteca* trees, I categorized the distance from *A. trigona* nests as the number of branching separations (or forks) from the *A. trigona* colonies. A distance of 1 indicates nest sets that were separated from *A. trigona* colonies by one or no forks, 2 indicates artificial nests that were separated by two forks, and 3 indicates nest sets that were separated from the *A.*

trigona colony by three or more forks (Figure 23). Artificial nests were checked for occupants during the following wet season and within *A. trigona* trees, I noted around which nest sets *A. trigona* workers came to artificial baits. If *A. trigona* came to baits within about 0.5 m from the nest sets, I considered the entire nest set to be within the territory range of *A. trigona*.

Ant Surveys

Ant surveys were conducted between the wet season months of June-September in 2018 and again in 2019 in the 19 *Dipteryx oleifera* trees with artificial nests. I additionally surveyed 9 more trees (6 with an *A. trigona* colony and 3 without) in 2019. I sampled ant workers using hand collections and bait. Baits were placed near the main fork and on all branches and lianas (woody vines) accessible from the main fork. Baits consisted of a mixture of carbohydrates (honey) and protein (chicken, ham, or tuna). Each bait was surveyed multiple times throughout the survey period as species turnover at individual baits is high.

I recorded ambient air temperature (°C), relative humidity (%), date, and time of day at the beginning of each survey. The survey period was limited to 60 minutes between the hours of 8:00-16:00. I collected and stored representatives of all ant species observed in 95% ethanol until identified using online and published keys (Ward 2003, Longino 2010). Identifications were confirmed with voucher specimens at the University of Louisville and the Smithsonian Tropical Research Institute.

Statistical Analyses

All data were analyzed in the R statistical environment (R Core Team 2019). To determine if occupancy of artificial nests differed between tree crowns with and without *A. trigona* colonies, I compared differences in the frequency of ant occupation in artificial nests, excluding nests that were occupied by *A. trigona*. The response term was a binomial variable (occupied or not occupied), *Azteca* presence or absence, nest type, and the number of days since nests were added into trees were the fixed effects, and tree identity (nested within treatment) was a random effect. I compared nested models with likelihood ratio tests to determine significance between trees with and without *A. trigona* and among nest types. I tested for differences between occupancy among nest types using a post-hoc Tukey HSD test. I used a Mann-Whitney U-test to determine if *A. trigona* ants occupied more nests in *Azteca* only trees than did other ant species. Additionally, I used a similar generalized mixed effect model approach to analyze if nest occupancy by ants was different in *Azteca* trees based on the categorical distance from the *A. trigona* nest, and whether nest occupancy was affected by the proximity of *A. trigona* foraging trails. Occupation by non-*A. trigona* ants was the binomial response variable, whereas categorical distance from the *A. trigona* nest, presence of *A. trigona* foragers, and all interactions were fixed effects. Tree identity was treated as a random effect.

I compared the species richness and composition of nest occupants in all nests, regardless of nest type (hereafter, *all nests*), and separately among the four artificial nest types (hereafter, *nest type*). For the *all nests* models, *A. trigona* presence was the binomial fixed effect and there was no random effect. For the *nest type* models, *A. trigona* presence, nest type, and the *A. trigona* presence by nest type interaction effect were fixed effects. Tree identity nested within treatment was included as a random effect. I compared species richness by treating the number of species that occupied artificial nests as the response variable. I used a linear model for *all nests* to determine the effect of *A. trigona* presence on overall species richness in the artificial nests (species richness was normally distributed), and a mixed-effect generalized linear model for *nest types* comparing nested models with likelihood ratio tests to analyze differences in species richness among nest type in trees with and without *A. trigona*.

I used PERMANOVA to evaluate differences in ant species composition in artificial nests between trees with and without *A. trigona* (*all nests*) and among artificial nest types (*nest type*). I calculated Bray-Curtis similarity matrices using incidence data and 9,999 permutations to determine p-values for each test. The resulting dissimilarity matrices were used to perform the PERMANOVA tests, PERMDISP tests, and ordination plots. I used a post-hoc pairwise PERMANOVA analysis to determine how species composition differed among nest types and post-hoc pairwise PERMDISP analyses to determine differences in beta diversity among nest types. I created classical multidimensional scaling (MDS) ordinations for *all nest* and *nest type* data, as stress values were too low to create an NMDS. I fit each species as a vector in each ordination to visually display which species best explained the separation of nest occupants along the axes. Additionally, I used indicator species analyses (*multipatt*, package indicspecies) to determine which species drove the differences in compositions shown by the PERMANOVA.

About a fourth of the artificial nests were destroyed (likely from termite damage), so I used a generalized mixed-effect linear model with stepwise model reductions to determine whether artificial nests in *A. trigona* trees were more likely to be destroyed. The response term was a binomial variable (destroyed or intact), while treatment, nest type, termite presence, and the interaction between treatment and termite presence were fixed effects. Tree identity (nested within treatment) was included as a random effect. I tested for differences among nest types using a post-hoc Tukey HSD test. I also tested for differences in artificial nest damage in *Azteca* only trees using nest type, categorical distance from the *A. trigona* nest, presence of *Azteca* foragers, and interaction between distance and *A. trigona* forager presence as fixed effects. As above, tree identity was included as a random effect.

I used similar methods as described above to determine whether the resident ant communities differed in trees with and without *A. trigona*. Specifically, I analyzed the species richness, composition, and beta diversity of all ants encountered in trees with and without *A. trigona* using data only from the 2019 surveys. The models treated the presence or absence of *A. trigona* as the fixed effect and there was no random effect. The number of species surveyed in total tree crowns were normally distributed, so I used a linear regression model (package *nlme*, function *lme*) to determine differences in species richness between trees with and without *A. trigona*. I conducted species co-occurrence analyses (*EcoSimR* package; Gotelli and Ellison 2013) to determine if there were patterns of species segregation and aggregation among ant species in *D. oleifera* tree crowns (i.e., ant mosaics; Sanders et al. 2007). Co-occurrence patterns explain levels of competition among species: larger occurrence values indicate that species are interspecifically competing (Gotelli 2000). I examined co-occurrence frequencies from individual tree crowns in all 28 trees between the control and *Azteca* treatments and across all tree crowns. I used the fixed-fixed model and generated 9,999 random matrices for each analysis.

Finally, to evaluate if artificial nests changed ant community parameters, I compared ant species composition before and after artificial nest installation in the 19 trees to which artificial nests were added. For each of these models, *A. trigona* presence or absence, year, and the interaction effect of *A. trigona* and year were the fixed effects and tree identity was the random effect. For the species richness model, I used a linear mixed-effects model with tree as the random effect.

RESULTS

Effects of A. trigona on artificial nest occupancy

About one fourth of the artificial nests were severely damaged (nests were missing or completely hulled out by insects; Figure 24) and I excluded these nests from the following analyses. Of the 283 remaining nests, 27 species of arboreal ants occupied 73 nests (Table 15, Figure 25); eight of these were occupied by *A. trigona*. Nests occupied by *A. trigona* workers were not considered in the following analyses unless otherwise stated.

There was no statistical difference in artificial nest occupancy between trees with and without *A. trigona*. However, there was a trend for fewer nests to be colonized in *A. trigona* trees. Specifically: 17.2 ± 2.96% nests were occupied among the 10 trees with *A. trigona* whereas 31.1 ± 4.26% nests were occupied among the 9 trees without *A. trigona* ($\chi^2 = 3.00$, df = 1, P = 0.083; Figure 26). The presence of *A. trigona* did not affect the number of species occupying all artificial nests ($F_{1,16} = 0.33$, P = 0.57) with an average of 2.00 ± 0.26 (2.4 ± 0.27 when *A. trigona* are included) and 2.78 ± 0.57 species occupying artificial nests in trees with and without *A. trigona*, respectively (Figure 27). Additionally, ant composition (*pseudo*-$F_{1,17} = 1.08$, P = 0.36) and beta diversity ($F_{1,16} = 3.53$, P = 0.078) in all artificial nests were similar between trees with and without *A. trigona* colonies (Table 16, Figure 28) and the presence of *A. trigona* had no effect on the frequency of artificial nests that were destroyed ($\chi^2 < 3.1$, P > 0.081 for *A. trigona* presence, termite presence, and the *A. trigona* by termite interaction).

Within a tree, the location of *A. trigona* foraging trails influenced the frequency of nest occupancy among the 10 trees with *A. trigona* colonies. In particular, artificial nests located within *A. trigona* foraging ranges were occupied less frequently than those located in non-*A. trigona* foraging areas ($\chi^2 = 10.59$, df = 1, p = 0.0011 for foraging; $\chi^2 < 0.64$, P > 0.72 for nest distance and the foraging by nest distance interaction effect). In particular, 30.5 ± 6.05% of nests outside of *A. trigona* foraging ranges were occupied, whereas only 8.7 ± 3.42% of nests were occupied within *A. trigona* foraging ranges. However, the location of *A. trigona* nests did not influence the frequency of destroyed nests ($\chi^2 < 3.87$, $P > 0.14$ for *A. trigona* foraging, *A. trigona* nest distance, and nest distance by foraging interaction) and *A. trigona* workers did not occupy a greater percentage of artificial nests than any other nest occupants (Mann-Whitney = 51, $P = 0.080$).

Effect of A. trigona on nest occupancy by nest type

Ants colonized the four types of artificial nests with similar frequency ($\chi^2 = 5.86$, df = 3, $P = 0.12$; Figure 26), regardless of *A. trigona* presence or the number of days nests had been in trees ($\chi^2 < 0.011$, $P > 0.42$ for *A. trigona* presence by nest type interaction and days since setup). Additionally, *A. trigona* presence had no effect on the number of species that occupied the different nest types ($\chi^2 < 2.1$, $P > 0.28$ for *A. trigona* presence and *A. trigona* presence by nest type interaction), although more species occupied cavity nests than occupied bark nests ($\chi^2 = 14.70$, df = 3, $P = 0.0021$; Table 17, Figure 29). The composition of nest type occupants was different between trees with and without *A. trigona* (*pseudo*-$F_{3,38}$ = 2.76, $P = 0.0024$; Figure 29a) and among the different artificial nest types (*pseudo*-$F_{1,38}$ = 1.80, $P = 0.0051$; Figure 29b), with no *A. trigona* by nest type interaction effect (*pseudo*-$F_{3,38}$ = 1.27, $P = 0.13$; Table 16). Indicator species analysis revealed that *Dolichoderus laminatus* was associated with bark nests in trees with *A. trigona* colonies (IndVal = 0.83, $P = 0.001$), whereas *D. bispinosus* was associated with cavity and bark nests in trees without *A. trigona* (IndVal = 0.70, $P = 0.003$). The composition of bark nesting species differed from both soil and twig nesting species whereas cavity nesting species composition differed from soil nesting species composition (Table 18, Figure 29b). However, beta diversity was lower in trees with *A. trigona* ($F_{1,37} = 7.12$, $P = 0.011$) and in bark nests ($F_{3,35} = 5.08$, $P = 0.0050$; Table 18). Some species showed trends of occupying different nest substrates in trees with and without *A. trigona*. For example, *Wasmannia rochai* occupied only soil nests in trees without

A. trigona and occupied each a soil, cavity, and twig nest in an *A. trigona* tree. Meanwhile, *Crematogaster crinosa*, a cavity nesting species that forms territorial boundaries with *A. trigona* in mangrove forests (Longino 2003), was found nesting only in a soil nest in this study. Finally, twig and bark nests were destroyed more frequently than cavity and twig nests ($\chi^2 = 168.72$, df = 3, $P < 0.001$ for nest type; $\chi^2 < 3.24$, $P > 0.71$ for termite presence, *A. trigona* presence, and *A. trigona* presence by termite presence interaction; Table 19).

Effects of A. trigona on resident ant assemblages

There were 66 species of ants collected in the 47 tree surveys among 28 *D. oleifera* trees (Table 15). Average (±SE) ant species richness (excluding *A. trigona*) did not differ between trees with (8.88 ± 1.04, n = 16) and without (8.67 ± 1.32, n = 12) *A. trigona* colonies ($F_{1,26} = 0.0059$, $P = 0.94$). Arboreal ant species composition (*pseudo*-$F_{1,27} = 1.71$, $p = 0.066$) and beta-diversity ($F_{1,26} = 0.20$, $P = 0.66$) also did not differ among trees with and without *A. trigona* colonies (Table 16, Figure 30). However, there were differences in the species co-occurrence patterns between the treatments. Whereas ant species distributions did not differ from random in the trees with *A. trigona* colonies, co-occurrence values were low in trees without *A. trigona* colonies (Table 20). Furthermore, among all 28 surveyed trees, co-occurrence values did not differ from random, and these values were similar when *A. trigona* were added into the models (Table 20).

Artificial nest addition did not affect the species richness ($F_{1,16} < 0.61$, $P > 0.44$ for *A. trigona* presence or absence, year, and *A. trigona* presence by year interaction effect), composition (*pseudo*-$F_{1,35} > 0.59$, $P > 0.065$ for *A. trigona* presence, year, and *A. trigona* presence by year interaction effect), or beta diversity ($F_{1,34} > 0.10$, $P > 0.19$ for both *A. trigona* presence and year) of the arboreal ant community in *D. oleifera* tree crowns.

DISCUSSION

Competition is considered the 'hallmark of ant ecology' and ecologically dominant species are expected to be the main drivers of ant community structure (Hölldobler and Wilson 1990, Lach et al. 2010). Here, I show that fewer nest resources are occupied when located in the foraging ranges of the ecologically dominant ant, *A. trigona*. Additionally, the species composition of nest occupants differs in trees with *A. trigona* colonies, suggesting that *A. trigona* affects local species distributions. However, the influence of *A. trigona* on the species composition of artificial nest occupants does not affect the total species composition within an individual tree crown. Ant community structure was not different between trees with and without *A. trigona*, suggesting additional support for the growing body of literature indicating that ant communities within more structurally complex forests are not shaped by competition (Ribas and Schoereder 2002, Blüthgen et al. 2004, Sanders et al. 2007).

Ants occupied artificial nests less frequently when they were clearly within active *A. trigona* foraging territories, suggesting that *A. trigona* limits access to resources under these circumstances. However, species composition between trees with and without *A. trigona* was similar, regardless of the potential effects of *A. trigona* on resource accessibility, contrary to the expectations of the ant mosaic hypothesis (Room 1971, Majer and Delabie 1993). One explanation is that the spatial heterogeneity in this canopy system mitigates the competitive exclusions observed in simpler systems due to greater resource availability (e.g. agroecosystems, the Brazilian cerrado, mangrove forests; Adams 1994; Philpott 2010; Powell et al. 2011). Ants occupied artificial nests at low frequencies in this study (< 25% of artificial nests) and it is likely that nest site availability is not a major limiting factor for ants in these *D. oleifera* trees. In fact, in this same system, occupancy of artificial cavity nests triples when nest and food resources (via lianas) are removed, and over half of occupied nests are by dominant species, including *A. trigona* (Adams et al. 2019). Thus, in more homogenous systems, patterns of species exclusions affecting the entire tree community may be a result of increased interactions with *A. trigona* which tend to occupy a greater area of the tree. In the *D. oleifera* trees used in this study, *A. trigona* trees had only one or two nests per tree and it is likely that ants were able to limit their interactions with *A. trigona* by avoiding their territories (e.g., using lianas as pathways to escape interactions, eavesdropping on pheromones to avoid workers - see Chapter 2 - and thus occupying resources outside of *A. trigona* territories).

Despite low frequency of total nest occupants, community composition of nest type occupants differed in trees with *A. trigona*. This shift in composition indicates that not all species respond equally to *A. trigona* and may provide some evidence that species partition nesting substrates when *A. trigona* is present. The varied nesting patterns of occupants in trees with and without *A. trigona* could indicate that *A. trigona* presence shifts nest type usage for some species, although due to the overall low frequency of nest occupancy, the power to detect such differences was limited in this study.

The composition of ants colonizing artificial nests in control trees was primarily associated with the presence of a different dominant species, *Dolichoderus bispinosus*. *D. bispinosus* presence could indicate that *A. trigona* and *D. bispinosus* have patterns of segregation as proposed by the ant mosaics hypothesis. However, given that the ant species surveyed in this study co-occurred randomly across all 28 *D. oleifera* trees, there is no evidence indicating that the nest usage by *D. bispinosus* corresponds with patterns of spatial mosaics. Furthermore, the 16 *A. trigona* trees showed no patterns of segregation whereas among the 12 trees without *A. trigona* colonies, species showed patterns of aggregation. Thus, patterns of exclusion by dominants appear to be contained within small regions of an individual tree crown. This result suggests that spatial mosaics shaping distributions in the entire tree crown may be the exception rather than the rule (Sanders et al. 2007).

Finally, the type of artificial nest was an important determinant of species occupancy in this study. I found that canopy ants occupied all nest types at similar frequencies. Additionally, I found that for some species the use of soil nests could provide evidence of niche shift when dominants are present. Most studies investigating canopy ant access to nest resources focus only on the use of cavity nests (Philpott 2010, Powell et al. 2011, Adams et al. 2019, Mottl et al. 2020). Including only cavity nesting substrates ignores over 30% of the nesting species in this study and the possibility that nesting strategies for some species, such as *C. crinosa*, might drastically shift under certain pressures. Therefore, the use of artificial nests for soil inhabiting species is necessary to evaluate more full-scale community-wide effects when testing the ecological effects of ant access to nest resources.

The results of this study support the hypothesis that the dominant ant, *A. trigona*, limits access to nest resources, but contrary to my predictions, this influence is only detectable within a small spatial scale. As predicted by the ant mosaic hypothesis, I found patterns of species exclusions of *A. trigona* with other dominant species. However, contrary to the ant mosaic hypothesis, such effects do not appear to be the major factor influencing species distributions in this canopy system. Instead, the interplay between spatial heterogeneity and resource availability may be more important to canopy ant community structure, as suggested in other studies (Blüthgen and Stork 2007, Sanders et al. 2007). Future experimental studies that manipulate resource availability within similar sized tree crowns across a diversity of forest habitats (such as primary, secondary, and agroforestry forest systems), and that include a variety of nesting substrates, would further address if collectively spatial heterogeneity and resource availability drives observed patterns of competition. Additionally, territorial boundaries, foraging ranges, and behaviors of dominants with interspecific species should also be determined within individual tree crowns to assess the range and degree of their influence.

TABLES

Table 15. The 66 ant species collected in this study. Values under Azteca and Control are collection frequencies (percent occurrence in tree collections) from trees with *Azteca* (n=15) and trees without *Azteca* (n=13). Markings under Nests represent species that were found in artificial nests and letters correspond to type of nest within which species were found. B = bark nests, C = cavity nests, S = soil nests, and T = twig nests. Morphospecies are labeled as "sp#".

Subfamily	**Species**	**Azteca**	**Control**	**Nests**			
Dolichoderinae	*Azteca brevus*	6.67	0				
	Azteca flavigaster	6.67	0				
	Azteca forelii	0	7.69				
	Azteca instabilis	6.67	7.69				
	Azteca nigricans	0	7.69				
	Azteca pilosula	6.67	0				
	Azteca trigona	100	0	B	C	S	T
	Dolichoderus bispinosus	33.33	61.54	B	C	S	T
	Dolichoderus debilis	0	7.69		C		
	Dolichoderus laminatus	46.67	46.15	B	C		
	Tapinoma melanocephalum	0	7.69				
Ectatomminae	*Ectatomma tuberculatum*	13.33	7.69			S	
Formicinae	*Brachymyrmex australis*	6.67	7.69			S	
	Brachymyrmex pictus	13.33	7.69				
	Camponotus atriceps	6.67	0				
	Camponotus brettesi	40.00	30.77		C		T
	Camponotus brevis	0	7.69				
	Camponotus linnaei	60.00	69.23		C		
	Camponotus mucronatus	6.67	7.69				
	Camponotus novogranadensis	26.67	30.77		C		
	Camponotus pittieri	6.67	0				
	Camponotus senex	53.33	15.38		C		T
	Camponotus sericeiventris	6.67	15.38				
	Camponotus sp.1	6.67	0			S	
	Camponotus sp.2	0	7.69				
	Camponotus textor	13.33	30.78		C		
	Nylanderia steinheili	6.67	0				
Myrmicinae	*Cephalotes atratus*	60	38.46				
	Cephalotes basalis	66.67	38.46				
	Cephalotes foliaceus	6.67	0				
	Cephalotes grandinosus	6.67	0				
	Cephalotes maculatus	60.00	23.08				
	Cephalotes minutus	26.67	7.69				

	Cephalotes umbraculatus	46.67	0	C		
	Crematogaster brasileinsis	13.33	0			
	Crematogaster carinata	0	7.69	C		
	Crematogaster crinosa	13.33	0		S	
	Crematogaster crucis	0	7.69			
	Crematogaster curvispinosa	20.00	7.69			
	Crematogaster limata	20.00	23.08	C		T
	Crematogaster rochai	6.67	7.69			
	Monomorium floricola	0	7.69		S	
	Pheidole caltrop	13.33	15.38	C		
	Pheidole harrisonfordi	6.67	7.69		S	
	Procryptocerus belti	20.00	15.38	C		
	Solenopsis JTL-012	6.67	0			
	Solenopsis picea	46.67	23.08			
	Wasmannia rochai	20.00	23.08	C	S	T
Ponerinae	*Neoponera carinulata*	6.67	7.69		S	
	Neoponera crenata	6.67	7.69			
	Neoponera foetida	33.33	23.08		S	
	Neoponera sp. 1	0	15.38			
	Neoponera villosa	60.00	61.54		S	
	Odontomachus sp. 1	6.67	0			
Pseudomyrmecinae	*Pseudomyrmex beccarii*	20	0			
	Pseudomyrmex boopis	6.67	0			
	Pseudomyrmex browni	0	23.08			
	Pseudomyrmex elongatus	40.00	30.77			
	Pseudomyrmex euryblemma	13.33	0			
	Pseudomyrmex gracilis	93.33	69.23	C		T
	Pseudomyrmex oculatus	60.00	61.54			T
	Pseudomyrmex simplex	6.67	15.38			
	Pseudomyrmex sp. 1	0	7.69			T
	Pseudomyrmex spiculus	6.67	0			
	Pseudomyrmex tenuissimus	60.00	23.08			
	Pseudomyrmex viduus	6.67	7.69			

Table 16. Significant correlations between NMS and MDS axis components and the species that occupied total trees, all artificial nests, and the different nest types. The R^2 value is proportional to the length of the vector in the ordination and ordination plots only show vectors with significant p-values.

SPECIES	TOTAL TREE				ALL NEST OCCUPANTS				NEST TYPE OCCUPANTS			
	NMS1	NMS2	R^2	P	MDS1	MDS2	R^2	P	MDS1	MDS2	R^2	P
Camponotus brettesi	0.640	-0.769	0.354	0.003								
Camponotus novogranadensis	0.447	0.894	0.256	0.026								
Camponotus sp.1	-0.279	0.960	0.228	0.044								
Crematogaster limata	-0.980	0.197	0.250	0.027	0.903	0.429	0.765	0.003				
Dolichoderus bispinosus	-0.895	0.447	0.667	0.002					-0.981	0.194	0.967	0.001
Dolichoderus laminatus	-0.530	0.848	0.391	0.001	-0.325	0.946	0.444	0.014	0.0184	-0.100	0.172	0.031
Neoponera villosa	0.757	0.654	0.665	0.004								
Procryptocerus belti					0.143	-0.990	0.528	0.015				
Pseudomyrmex elongatus	0.486	-0.874	0.200	0.051								
Pseudomyrmex gracilis	0.915	-0.403	0.266	0.023								
Pseudomyrmex oculatus	-0.927	0.376	0.266	0.021								
Pseudomyrmex tenuissimus	0.272	-0.962	0.418	0.001								
Wasmannia rochai									0.329	0.944	0.903	0.001

Table 17. Pairwise comparison of ant species richness among the four artificial nest types. Upper cell values are the z-values and lower cell values are the P-values from the Tukey post-hoc test. Bolded P-values represent significant differences between nest types.

	Cavity	**Soil**	**Twig**
Bark	3.09 **0.010**	2.27 0.10	1.05 0.71
Cavity		-1.12 0.67	-2.39 0.076
Soil			-1.38 0.50

Table 18. Pairwise comparison of the species composition distances from the centroid for nest type occupants (excluding nests occupied by *Azteca trigona*) in four artificial nest types in trees with and without *A. trigona* colonies. Upper cell values are the F-values and lower cell values are the P-values from pairwise PERMANOVA and PERMDISP tests. Bolded P-values represent significant differences between nest types.

	PAIRWISE PERMANOVA			**PAIRWISE PERMDISP**		
	Cavity	**Soil**	**Twig**	**Cavity**	**Soil**	**Twig**
Bark	1.72 0.086	2.65 **0.012**	2.17 **0.036**	0.14 **0.017**	0.16 **0.005**	0.17 **0.007**
Cavity		2.11 **0.018**	0.91 0.54		0.024 0.89	0.029 0.86
Soil			1.29 0.18			0.005 1.00

Table 19. Pairwise comparisons of frequency of destroyed artificial nests among the four artificial nest types. Upper cell values are the z-values and lower cell values are the P-values from Tukey post hoc tests. Bolded P-values represent significant differences between nest types.

	Cavity	**Soil**	**Twig**
Bark	-5.30 **<0.001**	-0.528 **<0.001**	-1.89 0.21
Cavity		0.017 1.00	4.73 **<0.001**
Soil			4.70 **<0.001**

Table 20. Results of species co-occurrence tests between trees with (*A. trigona*) and without (Control) *A. trigona* presence and among all 28 *Dipteryx oleifera* ant surveys without *A. trigona* (All - *A. trigona*) and with *A. trigona* (All) included in the model. Values in the observed ≤ and ≥ simulation cells are the respective p-values from co-occurrence tests. Bolded values represent significant differences between observed and simulated C-scores.

Test	**Observed C-Score**	**Simulated C-Score**	**Variance of Simulations**	**Observed ≤ Simulated**	**Observed ≥ Simulated**
A. trigona	3.89	3.89	<0.01	0.50	0.50
Control	2.41	2.48	0.01	**0.014**	0.99
All - *A. trigona*	7.14	7.17	<0.01	0.37	0.64
All	3.79	3.80	0.002	0.43	0.57

FIGURE LEGENDS

Figure 20. The author secured in a tree crown during an ant survey. Photo credit = D. Prince.

Figure 21. Schematics of the four different artificial nest types used in this study: a) twig nests, b) soil nests (dashed line indicates amount of soil added), c) bark nests, and d) cavity nests (dark grey circle indicates where cavities were plugged with glue and wood shaving). Nest sizes are scaled to each other.

Figure 22. The set-up of artificial nests in *Dipteryx oleifera* trees. White circles surround artificial nests in one of the five nest sets that correspond to the zoomed in nests in the bottom right corner. The arrow at the top points to an *A. trigona* nest. *a* = bark nest, *b* = soil nest, *c* = twig nest, and *d* = cavity nest.

Figure 23. The categorical distance of nest sets from *A. trigona* nests based on number of branching forks. Categorical distances represented by a) the branch or trunk distance and b) the artificial nest set distances from the *A. trigona* nest. Line type represents fork categories as such: *solid lines* = branch with *A. trigona* nest, *dashed lines* = branches or artificial nest sets categorized as distance of 1, *dotted lines* = branches or artificial nest sets categorized as distance of 2, *dot-dashed lines* = branches or artificial nest sets categorized as distance of 3. Circles surround *A. trigona* nests and boxes surround artificial nest sets.

Figure 24. Examples of destroyed artificial bark (a) and twig (b) nests.

Figure 25. Examples of artificial nests that were occupied: *Dolichoderus laminatus* occupy the upper layer of a bark nest (a), *D. bispinosus* exiting the cavity nest entrance to forage at bait (b), a *Neoponera villosa* colony nests under the leaf layer in a soil pot (c), and a *Camponotus brettesi* major worker at a twig nest entrance (d).

Figure 26. The percentage of total non-*A. trigona* ant occupancy in the 283 non-damaged artificial nests with 95% Clopper-Pearson confidence intervals. Filled circles refer to *Azteca* trees and open circles refer to control trees.

Figure 27. The average number of ant species that occupied 280 artificial nests in trees with (filled circles) and without (open circles) *A. trigona* colonies with 95% Clopper-Pearson confidence intervals. *Total* = average number of all occupant species per tree.

Figure 28. Multidimensional scaling of all occupants in artificial nests in control versus *Azteca* trees. Open symbols represent the composition in control trees whereas filled symbols represent composition in *Azteca* trees. Ellipses represent the 95% confidence intervals: *solid line* = *Azteca* trees and *dashed line* = control trees. Vectors represent significant associations between ant species and the corresponding points in the matrix. The length of the vector represents the strength of the association.

Figure 29. Multidimensional scaling of a) nest type occupants in control versus *Azteca* trees and b) among the four different nest types. Open symbols represent the composition in control trees whereas filled symbols represent composition in *Azteca* trees (a). Symbol type and color represent different types of artificial nests (b). Ellipses represent the 95% confidence intervals. Top panel ellipses: *solid line* = *Azteca* trees and *dashed line* = control

trees. Bottom panel ellipses: colors correspond to nest type colors. Vectors represent significant associations between ant species and the corresponding points in the matrix. The length of the vector represents the strength of the association.

Figure 30. Non-metric multidimensional scaling ordination of arboreal ant communities in 15 trees with (excluding *Azteca trigona* presence; filled circles) and 13 trees without (open circles) *A*. *trigona* colonies. Data are based on the combination of ant surveys that spanned across two years. Ellipses represent the 95% confidence intervals from the distance to the centroid of each treatment: *solid line* = *Azteca* trees and *dashed line* = control trees.

FIGURES

Figure 20.

Figure 21.

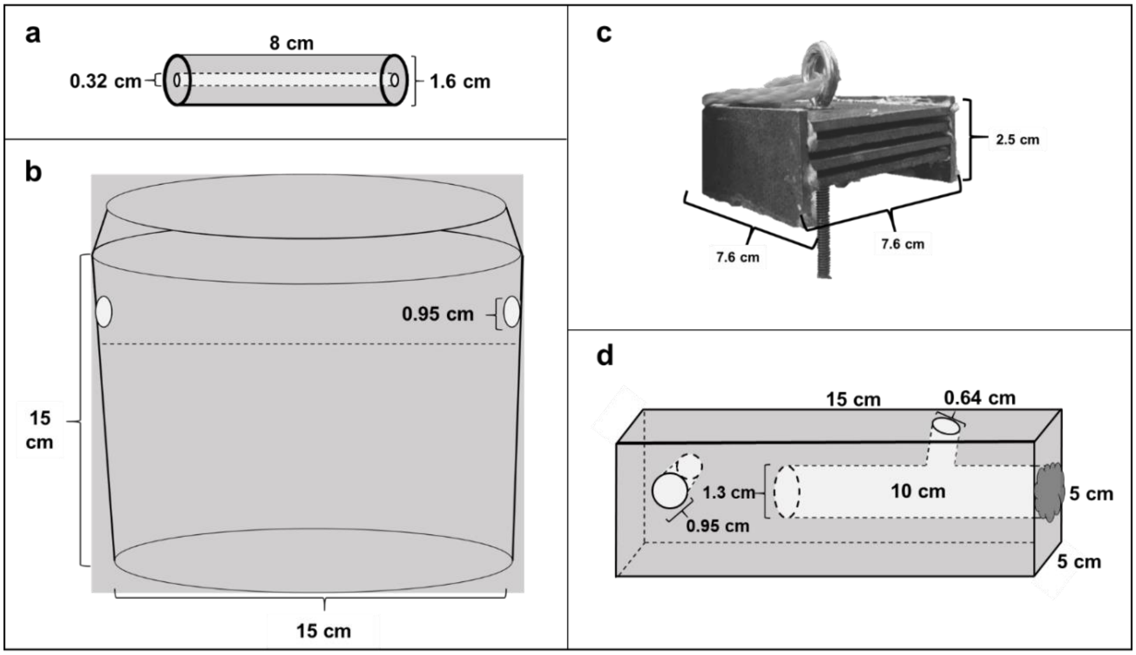

a
8 cm
0.32 cm
1.6 cm
b
0.95 cm
15 cm
15 cm
c
2.5 cm
7.6 cm
7.6 cm
d
15 cm
0.64 cm
1.3 cm
10 cm
5 cm
0.95 cm
5 cm

Figure 22.

Figure 23.

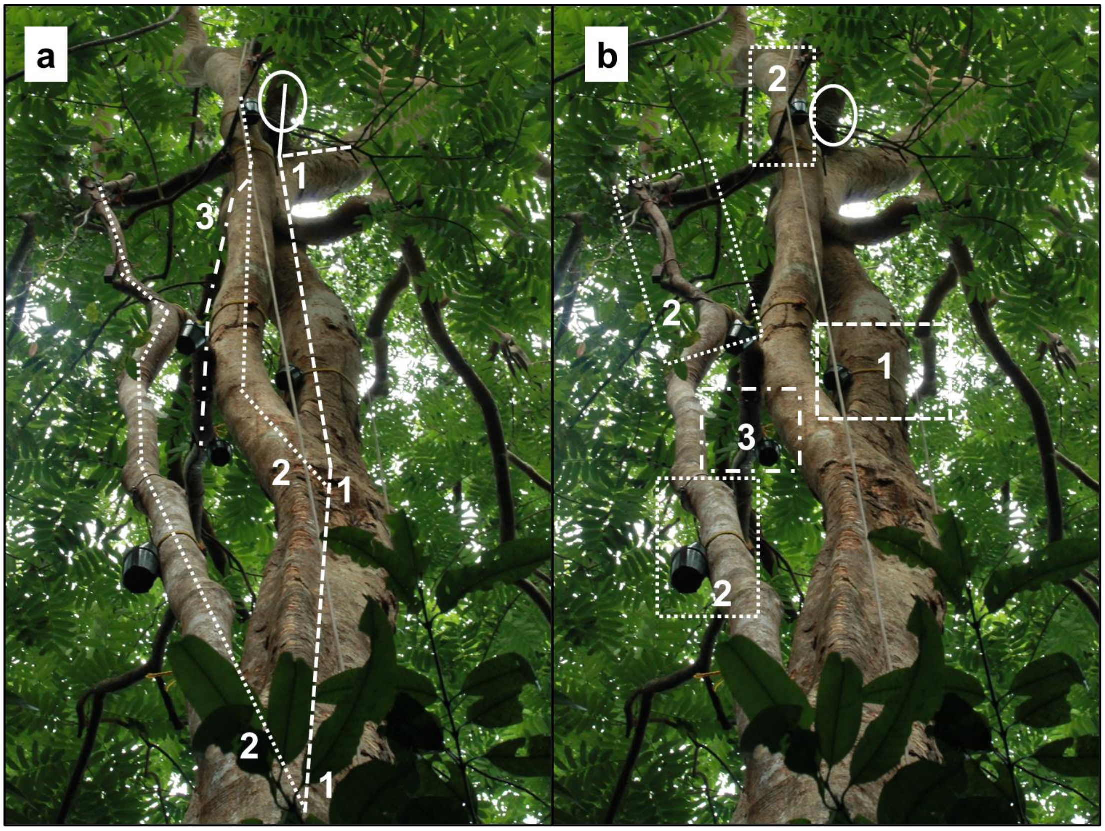
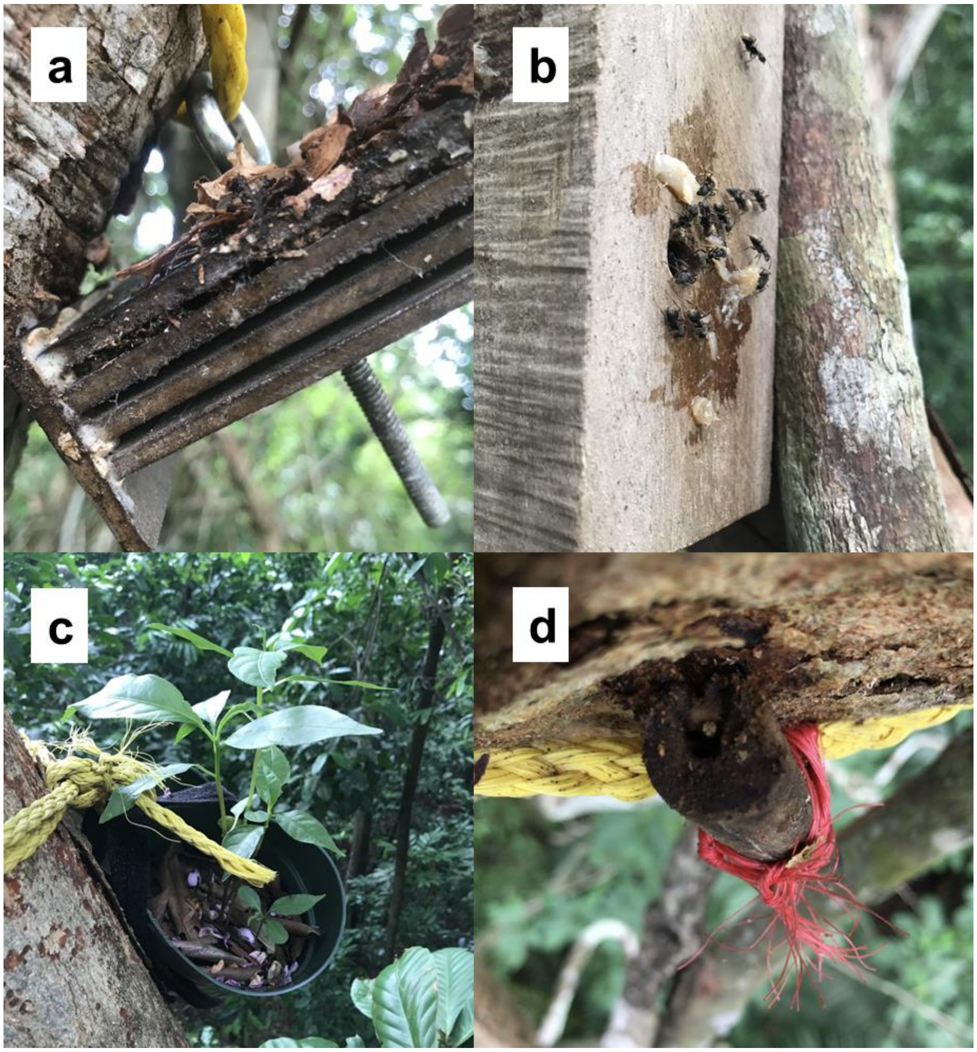


Figure 24.

Figure 25.



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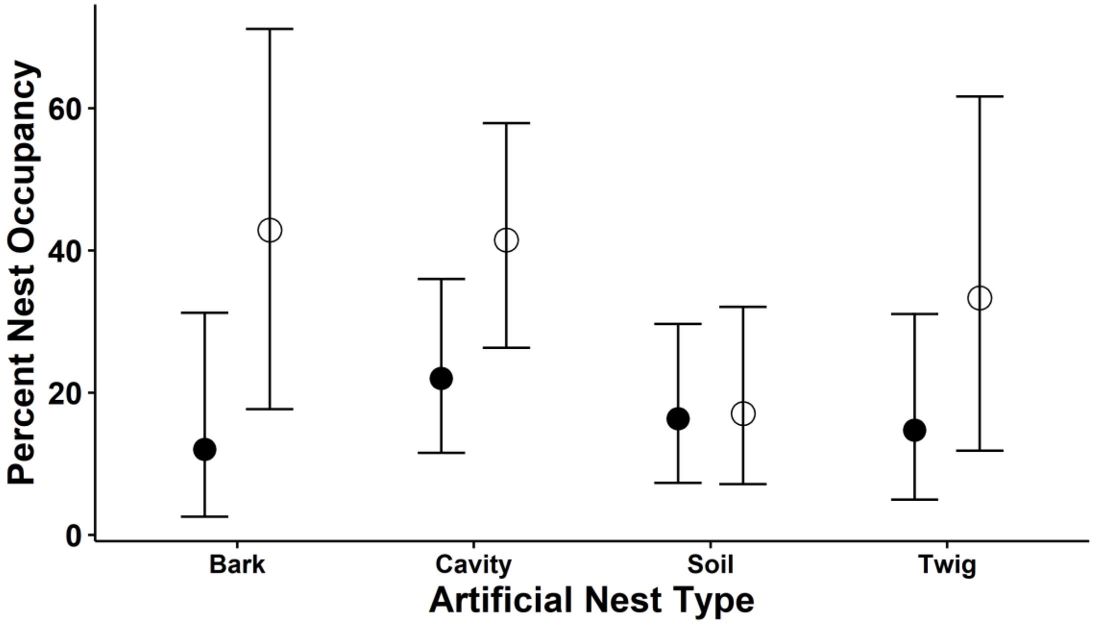


Figure 27.

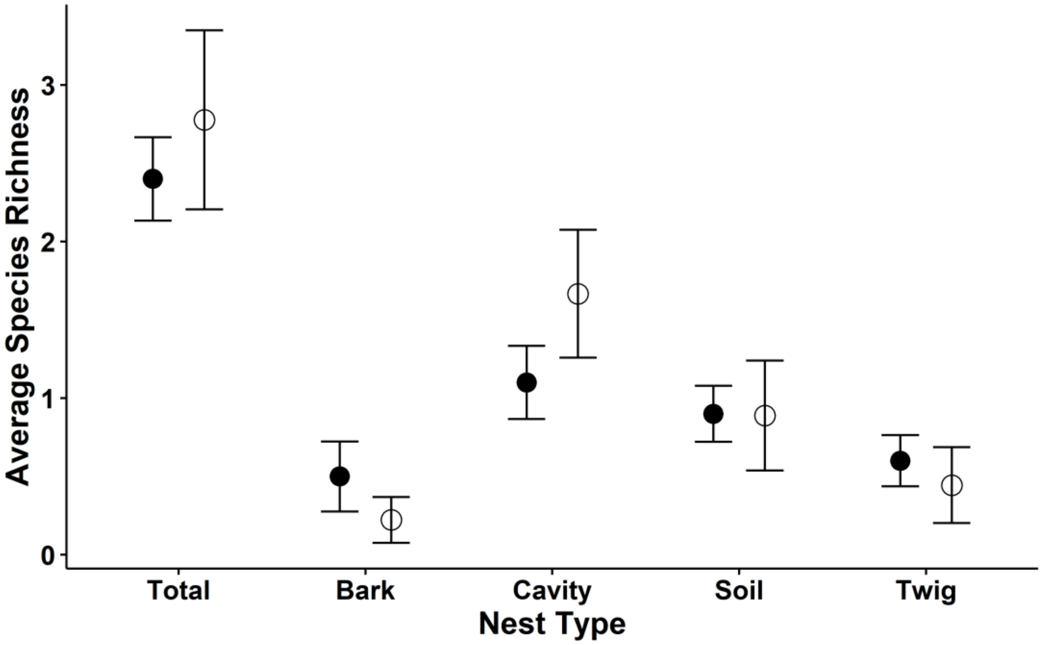
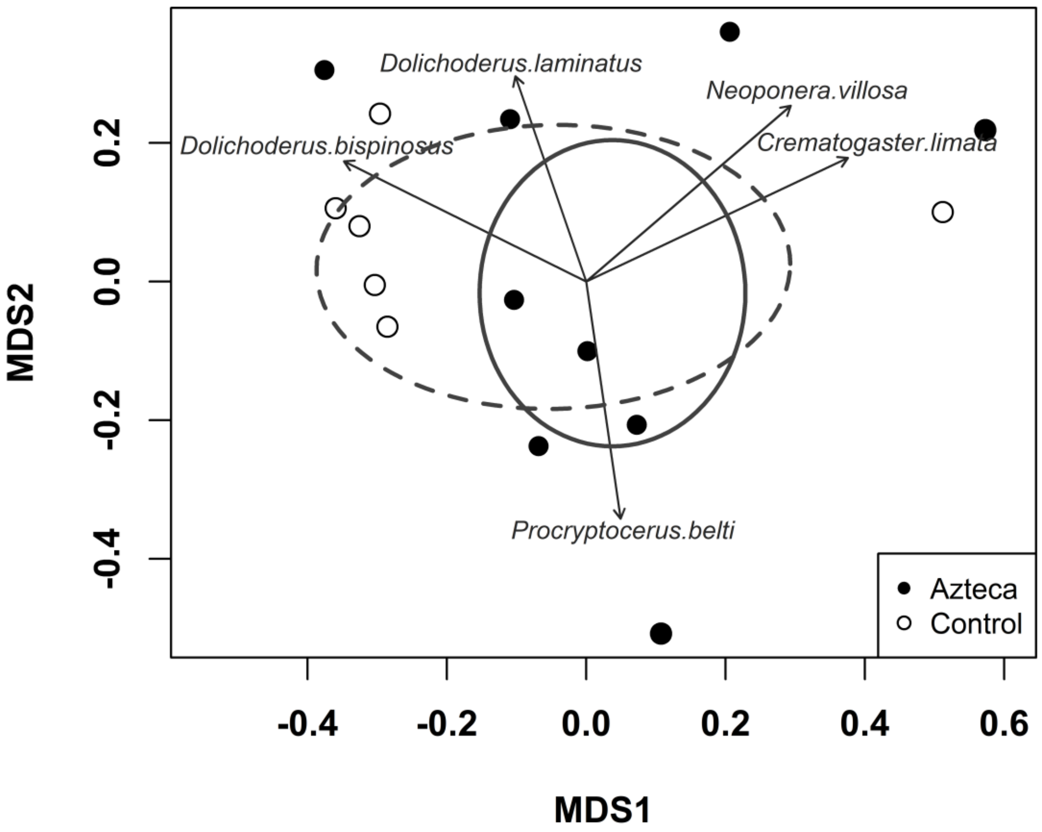


Figure 28.



Dolichoderus.laminatus
Neoponera.villosa
Dolichoderus.bispinosus
Crematogaster.limata
Procryptocerus.belti
● Azteca
○ Control
MDS2
0.2
0.0
-0.2
-0.4
-0.4
-0.2
0.0
0.2
0.4
0.6
MDS1

Figure 29.

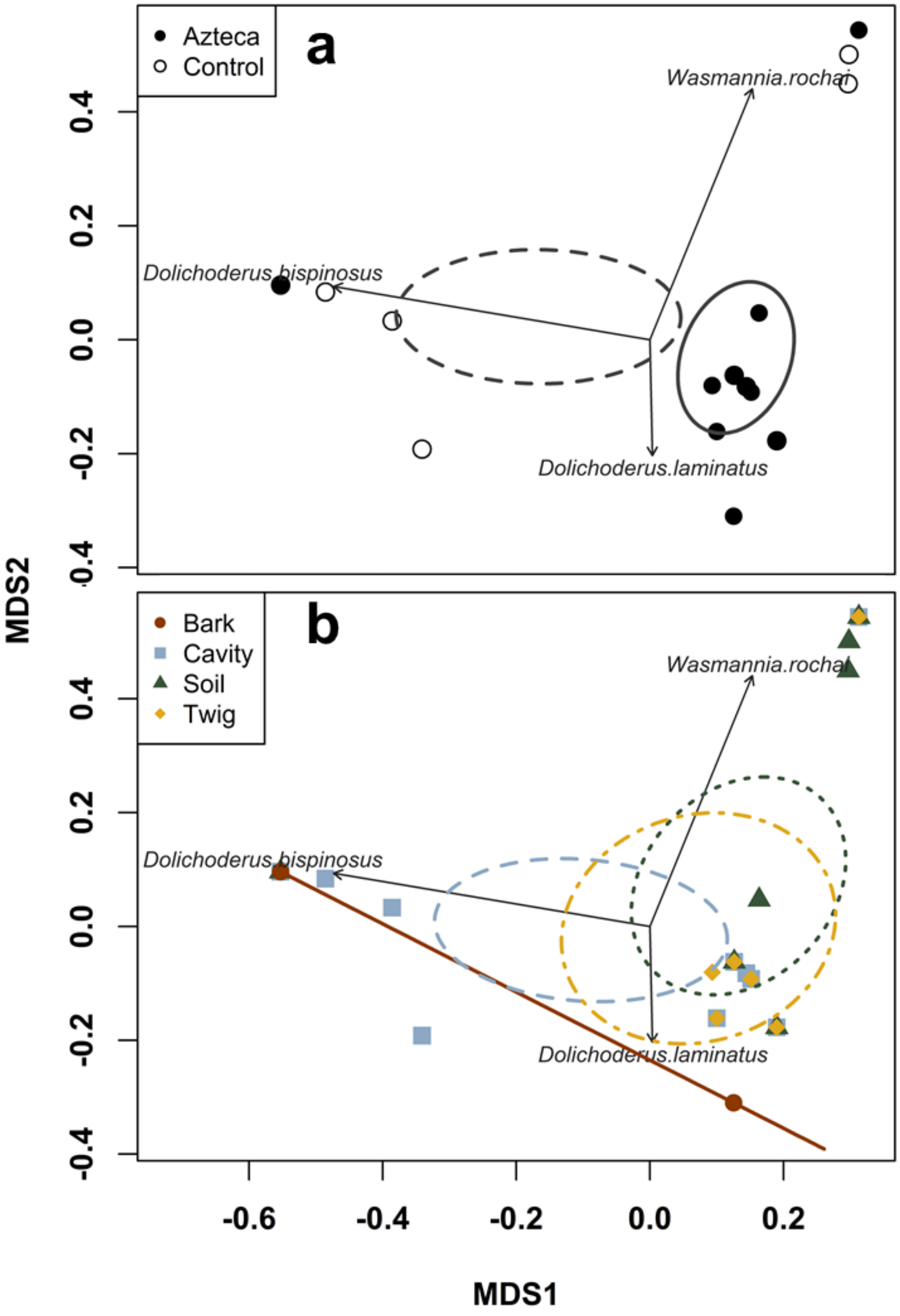
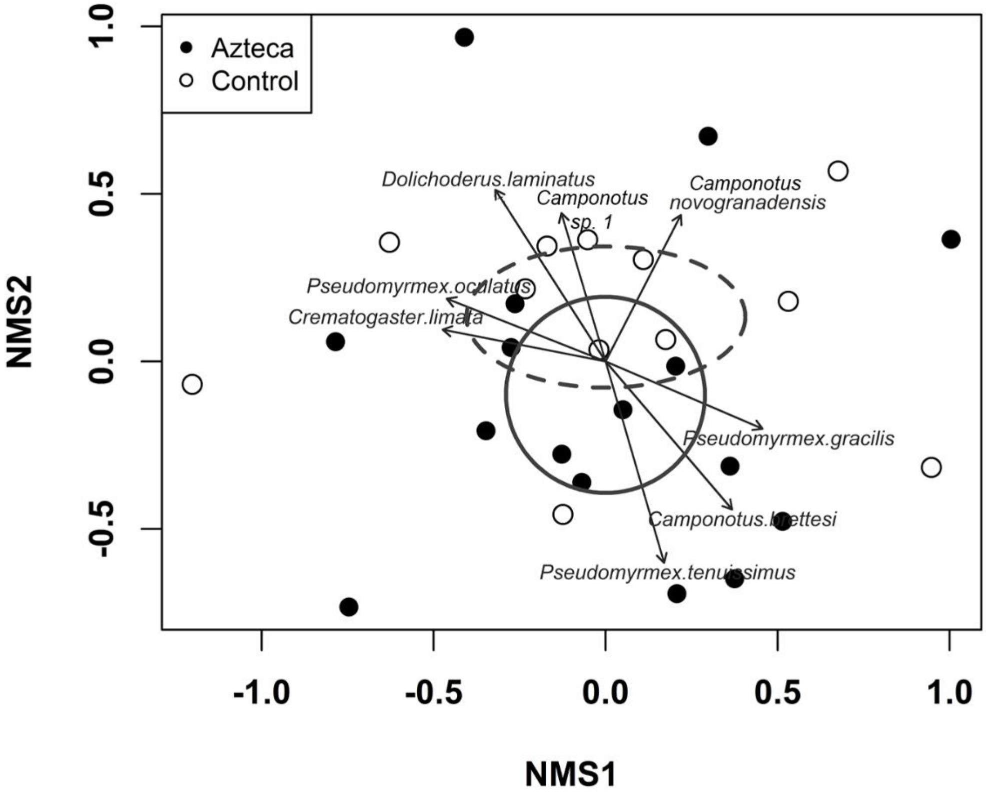

a
● Azteca
○ Control
Wasmannia.rochai
Dolichoderus.bispinosus
Dolichoderus.laminatus
b
● Bark
■ Cavity
▲ Soil
◆ Twig
Wasmannia.rochai
Dolichoderus.bispinosus
Dolichoderus.laminatus
MDS2
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.4
-0.2
0.0
0.2
MDS1

Figure 30.



● Azteca
○ Control
Dolichoderus.laminatus
Camponotus sp. 1
Camponotus novogranadensis
Pseudomyrmex.oculatus
Crematogaster.limata
Pseudomyrmex.gracilis
Camponotus.brettesi
Pseudomyrmex.tenuissimus
NMS2
1.0
0.5
0.0
-0.5
NMS1
-1.0
-0.5
0.0
0.5
1.0

CHAPTER V

DISTRIBUTION PATTERNS OF THE NEOTROPICAL ANT, *AZTECA TRIGONA*, IN THE BARRO COLORADO NATURE MONUMENT

SUMMARY

Landscape-level variation in the abundance of common species is an important driver of local diversity and ecosystem functioning. Ants are abundant in tropical forest canopies, yet the factors that shape canopy ant distributions at large spatial scales are unknown for most species. Here, I determined how the density of *Azteca trigona* nests varies with forest characteristics, topography, and edaphic properties at the landscape scale in a lowland forest of Panama. I also examined how *A. trigona* nest densities are affected by large-scale, long-term experimental liana (woody vine) removals. The density of *A. trigona* nests slightly decreased with increasing canopy height in younger forests, but increased with increasing canopy height in older forests. Additionally, soil type associated with texture and pH, geological formation, and liana removal affected the density of *A. trigona* nests. *Azteca trigona* nest densities were higher in liana removal plots than in control plots, but nest sizes were smaller in liana removal plots. The type of soil was the only predictor of nest size among the landscape variables. Collectively, this work demonstrates that canopy ant abundances may be affected by forest characteristics other than tree size or tree resources, and *A. trigona* densities are likely affected by a combination of forest properties, including edaphic and topographical properties. *Azteca trigona* is a common canopy species that can aggressively exclude co-occurring species from accessing resources and understanding where

these ants are most abundant can help us make predictions about species interactions and ecosystem processes in tropical forest canopies.

INTRODUCTION

One of the major goals of ecology is to understand what factors influence the distribution and abundance of organisms (Rosenzweig 1995, Volkov et al. 2003). Abundant, widespread species (i.e., common species) are often important drivers of local community structure and ecosystem functioning (Hillebrand et al. 2008, Grman et al. 2010, Sasaki and Lauenroth 2011). Understanding the factors that affect the distributions of common species provides a basis for predicting changes in community structure and ecosystem processes over time. Factors such as resource availability, climatic conditions, and habitat structure collectively influence the abundance of a species (MacArthur 1965, Volkov et al. 2003). However, determining which factors are most important can be challenging in diverse ecosystems like rain forests and coral reefs (Volkov et al. 2007).

Arthropods are especially diverse in the tropical rainforest canopy and play key ecological roles as predators, herbivores, and mutualists in this setting (Basset et al. 2007, 2012). In particular, ants (Formicidae) account for up to 90% of the canopy arthropod biomass (Tobin 1995, Davidson and Patrell-Kim 1996). Moreover, arboreal ant biomass is often dominated by one or two common species that shape local community structure to varying degrees (Leston 1978, Davidson 1998, Dejean et al. 2007). Factors such as forest structure and biogeochemistry affect local variation in arboreal ant richness and the influence of common species (Powell et al. 2011, Adams et al. 2017, Bujan et al. 2019), but the combined effects of such factors on the landscape-level distribution of canopy ants is unknown for most species. Understanding these patterns is fundamental to understanding large-scale species distributions and ecosystem function (Majer et al. 1994, Philpott and Armbrecht 2006, Law and Parr 2020).

Tree characteristics such as size and connectivity are good indicators of species richness in tropical forests (Campos et al. 2006, Adams et al. 2017, Antoniazzi et al. 2021). For example, taller trees often have higher ant species richness because tree height is typically associated with area (Campos et al. 2006, Adams et al. 2017). Additionally, trees that are more structurally heterogenous often support more resources and species (Antoniazzi et al. 2021). Moreover, species richness is often greater in primary vs secondary forests, likely due to a higher proportion of larger trees in primary forests (Schonberg et al. 2004, Klimes et al. 2012, Hernández-Flores et al. 2021).

Although associations between ant community structure and tree characteristics have been relatively well-studied, the effects of other factors, such as edaphic properties, on arboreal ants are less clear. For example, soil texture, form, and nutrient content are important factors that shape litter ant communities (Vasconcelos et al. 2003, Boulton et al. 2005, McGlynn et al. 2009), yet similar patterns for arboreal ants are unknown. This is an important gap because soil texture and forest topography are important determinants of the composition of tree species, and tree species affect species richness and composition of arboreal ants in some systems (Ribas et al. 2003, Adams et al. 2017; but see Kaspari and Yanoviak 2001, Kaspari et al. 2008, Bujan et al. 2016). Additionally, edaphic properties such as soil nutrient content influence the abundance of sap-sucking insects (Neves et al. 2010), which are major components to many arboreal ant diets (Davidson et al. 2003, Blüthgen et al. 2004). Tracking the number and size of arboreal ant colonies over large areas is difficult, due to crown accessibility limitations. Thus, few studies have estimated how canopy ant colonies may be affected by multiple forest factors including soil type, canopy height, and liana presence.

Lianas (woody vines) are a key structural component of most tropical forests (Schnitzer and Bongers 2002). They increase the habitat complexity for cursorial insects and provide important resources specifically for ants, such as nesting sites (Yanoviak and

Schnitzer 2013), food (Blüthgen et al. 2000, 2004), and efficient foraging pathways (Yanoviak 2015, Adams et al. 2017, 2019). However, the relevance of lianas to any given ant species is unknown, and the mutualisms that occur between insects and other climbing plants apparently are uncommon for tropical ants and lianas (Yanoviak 2015). Given that lianas are a diverse growth form and infest greater than 70% of canopy trees in lowland forests, it is likely that their ecological effects are important, although not clearly defined.

The species complex *Azteca chartifex/trigona* (hereafter, *A. trigona*) is an ideal candidate for studying the effects of landscape-level variation on species abundances. The large, pendulous, carton nests built by *A. trigona* are easily observed from the forest floor (Figure 2), and the size of the carton nest is a reliable indicator of worker abundance (Wheeler 1986, Longino 2007).

Azteca trigona colonies are polydomous, typically consisting of a larger central nest (sometimes exceeding 2 m in total length) surrounded by multiple smaller satellite nests that vary in size down to <25 cm in total length (Wheeler 1986, Longino 2007). *Azteca trigona* also is a common arboreal species that plays key ecological roles in the forest (Adams 1994, Clay et al. 2013, Bujan et al. 2016). As a behaviorally dominant species, *A. trigona* also potentially shape local ant communities in some forests (Adams 1994, Ribeiro et al. 2013, Adams et al. 2017). In intact forests, *A. trigona* tend to nest in larger trees (Lucas et al. 2018, Bujan et al. 2019); however they are also abundant in highly disturbed sites like roadsides and urban parks (Longino 2007, *pers. obs.*). Despite the ecological importance of these ants, the main factors affecting their colony size and density along landscape-level gradients are unknown.

The main goal of this study was to determine how *A. trigona* nest densities vary with forest structure, topographical characteristics, and edaphic properties in a lowland forest of Panama. Secondarily, I explored the effects of experimental liana removal (Van Der

Heijden et al. 2015, García León et al. 2018) on *A. trigona* nest density and size at this site. Given that older forests tend to offer a greater diversity of resources for ants (Klimes et al. 2012), I predicted that the density and size of *A. trigona* nests would increase with forest age and tree height. I also expected that liana removal would decrease *A. trigona* nest density by constraining the available foraging territory of each colony to a single tree and nearby ground-based resources.

METHODS

Fieldwork was conducted within the Barro Colorado Nature Monument (BCNM; 09.15°N, 79.85°W) from 2010 to 2019. The BCNM is a lowland, seasonally moist tropical forest with a wet season spanning from May to December (see Croat 1978; Leigh et al. 2014 for further site descriptions) and *A. trigona* is common in the forest canopy here.

BCI Survey

To explore how the density of *A. trigona* nests varies at the landscape scale on Barro Colorado Island (BCI), I surveyed the number of carton nests observed along each of the 27 BCI trails and associated shortcuts in 2019 (Figure 31). I conducted point counts for *A. trigona* nests every 30 meters along each trail, starting at the trail head and taking a final point count at the trail end. I counted the number of nests that were observable at each point and recorded both the number and size of nests. The amount of time spent in any given point varied with local forest complexity, but was limited to a maximum of 2 minutes. I assigned each nest to one of four size categories based on estimated nest length: <25 cm, 25-50 cm, 50-100 cm, and >100 cm. Nests that were visible from more than one observation point were only counted once. I recorded the location of each point as the distance from the nearest trail marker (trail markers exist every 100 m along each trail). The latitude and longitude coordinates of each point were subsequently determined with Garmin BaseCamp software. The number and size of nests were loaded into ArcGIS software with their corresponding coordinates. I estimated the total number of *A. trigona* nests on BCI by extrapolating from the total area observed along the trails assuming that each point count covered a 15 m radius.

I quantitatively evaluated the association between *A. trigona* nest density and variation in multiple landscape factors including soil type, canopy height, ground slope, forest age, and local geological history. Geological formations provide the parent material to soils and are important determinants of soil nutrient availability and texture (Baillie et al. 2007, Schreeg et al. 2010). BCI is composed of four major geological formations spanning from the early Oligocene through the early Miocene (Figure 31a), four common forms of soil comprised of fine loam topsoil and heavy clays (Figure 31b; Baillie et al. 2007), and a mixture of secondary (80-150 years old) and old growth (about 400 years old) forests (Figure 31c; Enders 1935). Additionally, the topography on BCI is comprised of mostly gentle slopes (<8° of sloping; Figure 31d) and a forest canopy that ranges from 0-50 meters in height (Figure 31e; Lobo and Dalling 2013). Forest variable information was accessed from the Smithsonian Tropical Research Institute GIS portal (stridata-si.opendata.arcgis.com) and downloaded into ArcGIS software. The information contained within these GIS datasets were created or collected from maps, historical records, and LiDAR overflights (Enders 1935, Stewart et al. 1980, Baillie et al. 2007). Canopy height was measured as the difference between the ground and surface elevations from LiDAR Digital Terrain and Digital Surface models that were collected 2000 m above ground level with 1 m^2 pixels (Mascaro et al. 2011). Degree of slope was calculated with 1 m^2 spatial resolution from the LiDAR Digital Terrain sum of slopes information (Lobo and Dalling 2013).

All analyses were conducted in R studio. I used a generalized linear regression model (*lme4* package) to determine what landscape variables affect variation in the density and size of *A. trigona* nests on BCI. The model included the soil type (brown loam, heavy clay, pale swelling clay, and red heavy clay) geological formation (Andesite, Bohio, Marine facies, and Volcanic facies), forest age, canopy height, slope, and the biologically relevant interaction effects (Table 21) as fixed effects. The continuous variables (canopy height and slope) were checked for normality and the cubed root of the degree of slope was included in the model. I conducted a generalized linear model on negative binomial data. The best fit model was determined via backwards model reduction based on AIC values (Table 21). I tested differences between any significant terms with a Tukey post-hoc analysis.

To evaluate if nest sizes varied across the landscape variables, I treated the number of nests as the response term for Poisson distribution and the landscape variables as the fixed effects. I conducted a generalized linear model that included only point locations that had *A. trigona* nests. The number of nests was the response variable, the categorical size of nests (as described above) and the landscape variables listed above were the fixed effects, and size by each individual forest factor were included as additional interaction effects.

Liana Removal Surveys

I explored the role of liana presence (via manipulation of liana abundance) on *A. trigona* presence in sixteen 80 x 80 m plots on the Gigante Peninsula (Figure 32). Half of these plots had all lianas removed in 2011 and have since been managed for no new liana growth (Van Der Heijden et al. 2015, García León et al. 2018). The number of *A. trigona* nests were counted in each plot along four transects that were spaced 20 m apart. Scans for *A. trigona* were conducted every 20 m along each transect (for a total of 16 scans per plot) by observing the forest vegetation from eye-level up to the canopy (Figure 32c). Plots were surveyed in 2010 prior to liana removals and yearly post removal from 2011-2019 (excluding years 2016 and 2018). Due to time constraints, two removal plots and one control plot were not surveyed in 2011 and two control plots and one removal plot were not surveyed in 2015. I additionally recorded the size of nests that were observed within the 16 plots during the 2019 survey (size was documented as described above).

I compared the number of *A. trigona* nests in liana removal and control plots pre and post liana removal. I used a generalized mixed-effect linear regression model to determine if the density of *A. trigona* nests was affected by liana removals across the nine years of surveys. The number of nests was the response variable with a Poisson distribution; treatment, year, and the treatment by year interaction were fixed effects, and plot nested within treatment was a random effect. To assess if *A. trigona* nest sizes changed after liana removal, I compared the size of nests observed in the final survey between liana removal and control plots. I used a generalized linear model, treating the number of nests as the response term, nest size based on the four nest size categories listed above, treatment, and the interaction between nest size and treatment as the fixed effects.

Finally, to establish if *A. trigona* colony sizes differed between treatments, I compared the number of colonies and the number of workers between liana removal and control plots with two T-tests. Given that *A. trigona* are polydomous, I estimated the number of colonies per plot by assuming the following: nests < 25 cm are 25% of a colony, between 25-50 cm is 50% of a colony, between 50-100 cm is 75% of a colony, and > 100 cm is one complete colony (100%). I estimated the number of workers based on worker abundance measurements from a 25 cm long x 15 cm wide x 9 cm thick *A. trigona* nest (28,000 workers; Wheeler 1986). I estimated the number of workers per size classes by multiplying the smallest length per size class (using 5cm for the < 25 cm long nests) by 15 cm width and 9 cm thickness. I estimated 5,600 workers in nests < 25 cm, 28,000 workers in nests 25-50 cm, 56,000 workers in nests 50-100 cm, and 112,000 workers in nests > 100 cm (Table 22).

RESULTS

BCI Surveys

I found 714 *A. trigona* carton nests along 38.6 km of trails on BCI with an average of 0.71 ± 0.04 nests observed per point count (Figure 31). More than half of the observed nests were greater than 50 cm long (Table 22). I estimated that there are more than 15,000 *A. trigona* nests on BCI, comprising over 490 colonies that house more than 195 billion workers (although worker estimates are likely greatly underestimated).

The density of *A. trigona* nests was affected by a variety of forest factors. The presence of *A. trigona* nests was influenced by the interaction effect of age by height (χ^2 = 4.56, df = 1, P = 0.033; Figure 33a), by the geological formation of the forest (χ^2 = 22.20, df = 3, P < 0.001; Table 23), and by the soil type (χ^2 = 18.52, df = 3, P < 0.001; Table 23). All other effects in the model did not affect nest densities (χ^2 < 4, P > 0.12) except for canopy height (χ^2 = 4.71, df = 1, P = 0.030). Nest densities were greater on the Volcanic and Marine facies than on the Bohio and Andesite formations (Figure 33b) within the red heavy clay versus the brown fine loam (Table 23, Figure 33c). The number of the different sized nests was affected by the size-by-soil interaction effect (χ^2 > 26.15, df =9, P = 0.002; Table 24, Figure 34).

Liana Removal Surveys

The removal of lianas had an effect on the density of *A. trigona* nests. There was a slight interaction effect of year by treatment on *A. trigona* nest density (χ^2 = 3.64, df = 1, P =

0.057), however nest density was greater in liana removal plots ($\chi^2 = 3.95$, df = 1, P = 0.047) and increased in all plots over time ($\chi^2 = 224.0$, df = 1, P < 0.001; Figure 35). *Azteca trigona* nest density increased on average by 1.36 ±0.23 nests in all 16 forest plots over the nine years of surveys. In the final survey, there were 12.25 ±4.3 *A. trigona* nests on average in removal plots and 9.26 ±3.3 nests in control plots. About 55% of *A. trigona* nests in liana removal plots were less than 50 cm in length whereas about 63% of nests were greater than 50 cm in length in control plots. The number and size of nests observed among the plots eight years after liana removal also differed ($\chi^2 = 15.89$, df = 3, P = 0.001; Figure 36). However, the estimated number of colonies was similar between control and removal plots (t = 0.96, df = 10.1, P = 0.36), although control plots were estimated to have over 790,000 more *A. trigona* workers on average than liana removal plots (t = 3.89, df = 7.2, P = 0.006; Table 22).

DISCUSSION

Disentangling the major factors that influence the abundance of an organism across the landscape can be challenging within diverse ecosystems such as a tropical forest. Here, I show that landscape-scale variation in the density of a common neotropical canopy ant is influenced by edaphic properties and liana presence within the Barro Colorado Nature Monument. Given that *A. trigona* are common within these forests and exhibit aggressive exclusion behaviors, understanding their distribution patterns is relevant to understanding broader aspects of forest ecology, including ecosystem-level processes.

Soil texture and nutrient content are derived from geological parent materials and it was unsurprising that *A. trigona* nest densities were influenced by both soil type and geological formation here (Baillie et al. 2006). Specifically, *A. trigona* nest densities were greater in the soil types that had higher silt content and were more acidic. Although it is unclear if these soil characteristics directly affect *A. trigona* densities, it is likely that the effects that these soil characteristics have on tree species composition and nutrient availability indirectly affects *A. trigona*. For example, soil nutrient availability, water retention ability, and soil pH levels are important factors determining tree species composition and plant quality (Coley et al. 1985, Bohlman et al. 2008, Schreeg et al. 2010). Given that *A. trigona* feed mainly on sap-sucking insect exudates (Davidson et al. 2003) and arboreal ant species richness and composition is influenced by tree species composition (Ribas et al. 2003, Adams et al. 2017), soil characteristics that directly influence tree species composition and sap-sucking insect exudates (Neves et al. 2010) likely have indirect effects on the success of *A. trigona* colonies. However, future studies are needed to reveal any indirect influences of soil type on *A. trigona* densities.

Azteca trigona nests in the pale swelling clay soil areas showed similar patterns as nests in liana removal plots (i.e., fewer nests larger than 50 cm long). Pale swelling clay soils are associated with areas of greater tree fall gaps (Lobo and Dalling 2013), suggesting that *A. trigona* colony growth is limited in disturbed areas. Forests with an even distribution of different sized nests may then be reflective of areas with higher frequencies of disturbance. Additionally, *A. trigona* nest densities were greater on smaller trees in younger forests and on larger trees in older forests. *A. trigona* nests tend to be larger when trees are larger and smaller when trees are in nitrogen addition plots (Bujan et al. 2019). Additionally, workers exhibit increased activity when phosphorous is increased in the soil (Bujan et al. 2016). Given that soils in younger forests often have greater phosphorous content and older forests have greater nitrogen content (Davidson et al. 2007), there may be a tradeoff for *A. trigona* to nest where soil phosphorous contents are greater and the ability to territorially maintain dominance over such resources may increase in these areas (i.e., in younger, less structurally complex tree crowns). When trees are more structurally complex, with greater abundance and diversity of resources, high levels of soil nitrogen may be inconsequential. However, experimental support is still needed to determine why *A. trigona* are more abundant under these forest parameters and furthermore, how their abundance influences heterospecific species.

Contrary to my predictions, *A. trigona* nest density increased in removal plots versus controls. *Azteca trigona* workers often scrape deadwood from the ground for nest construction (N. Clay, *pers. comm.*), and the increase in dead wood on the forest floor due to liana removal could explain the increase in nest numbers in removal plots. However, there was a greater frequency of smaller nests in removal plots. Lianas provide multiple resources to ants, such as pathways, food, and nesting sites (Blüthgen et al. 2000, Yanoviak 2015, Adams et al. 2019) and the reduction of these resources affects local species richness and composition (Powell et al. 2011, Adams et al. 2017, 2019), likely via competitive interactions (Adams 1994, Dejean et al. 2015). Although reduction of pathways could reduce the cost of maintaining territories (Eason 1992), the reduction of other resources is likely a greater cost that hinders the size of *A. trigona* colonies. The size of *A. trigona* colonies was likely inhibited over time (fewer resources available prevented colonies from growing in size) or immediately post liana removal (sudden depletion of many resources resulted in the loss of many individuals). Alternatively, given that the density of *A. trigona* nests increased in liana removal plots more than in control plots, the greater proportion of smaller nests in liana removal plots could be an effect of colonies partitioning workers to different sections of a tree. By doing so, *A. trigona* may be usurping resources in a greater proportion of a tree; such a potential effect may contribute to the patterns of mosaic like species exclusions observed in other simple canopy systems like agroecosystems and mangrove forests (Adams 1994; Dejean et al. 1999). However, more information is needed on species interactions under these circumstances.

This is one of the first studies to evaluate multiple landscape variables on the density and colony size of a common arboreal ant. The patterns observed here reveal that *A. trigona* colonies are a common component of the Barro Colorado Nature Monument, although colony sizes tend to be influenced by factors such as soil properties and liana presence. Understanding the mechanisms that shape the density and size of *A. trigona* colonies should provide more insight into the health or productivity of the forest alongside the species interactions and community parameters in the canopy.

TABLES

Table 21. Results of the stepwise model reduction of the generalized linear model used to test density of *A. trigona* nests across BCI. *A* = Forest age, *G* = Geological formation, *H* = Canopy height (m), *SL* = degree of slope, and *SO* = Soil type. AIC values (AIC) and the difference of AIC values between the model with the lowest AIC value (ΔAIC) are listed. Models within 2 ΔAIC are bolded. Forest variables in italics represent significant variables ($\alpha < 0.05$) in the model used for analysis.

Model	**AIC**	**ΔAIC**
A + *G* + H + SL + *SO* + *A:H* + H:SL	**2303.5**	**0**
A + G + H + SL+ SO + A:H + A:SO + H:SL	2305.8	2.3
A + G + H + SL+ SO + A:H + A:SO + H:SL + G:SO	2313.6	10.1
A + G + H + SL+ SO + A:H + A:SO + H:SL + G:SO + A:H:SO	2321.2	17.7

Table 22. The number of *A. trigona* nests and estimated worker numbers detected in 16 80 x 80 m liana removal forest plots on Gigante and along 38.6 km of BCI trails. Nest numbers are broken down into size categories based on nest length (cm). Worker numbers are estimated from number of workers in a 25 x 15 x 9 cm^3 nest (w). Values within nest size cells represent the average percentage of nests control and liana removal plots on Gigante and the total percent of nests along all trails on BCI. Worker numbers represent average number of workers between the Gigante removal plots and estimated workers along all 27 BCI trails.

		NEST SIZES				
Forest	**Location**	**XSmall** <25cm 5,600w	**Small** 25-50cm 28,000w	**Medium** 50-100cm 56,000w	**Large** >100cm 112,000w	**Worker Numbers**
Gigante	Control	17.89	18.70	23.58	39.84	984,900
	Removal	29.45	25.45	23.27	21.82	192,500
BCI	All Trails	17.65	21.85	26.89	33.61	$1.95*10^{11}$

Table 23. Pairwise comparison of BCI geological formations and soil types to the density of *A. trigona* nests. Upper cell values are the z-values and lower cell values are the P-values from Tukey post hoc tests. Bolded values refer to geological or soil types that differed.

GEOLOGY

	Bohio	Marine	Volcanic
Andesite	-0.26 0.994	**2.57** **0.048**	2.53 0.054
Bohio		**2.77** **0.028**	**3.23** **0.007**
Marine			-0.405 0.977

SOIL TYPE

	Heavy	Pale	Red
Brown	-1.29 0.543	0.99 0.738	**3.87** **<0.001**
Heavy		1.82 0.238	2.38 0.070
Pale			1.37 0.488

Table 24. Results of the stepwise model reduction of the generalized linear model used to test the density of *A. trigona* nests among sizes across BCI. *SI* = size of nest. See Table 21 label for table information.

Model	AIC	ΔAIC
SI + SO + SI:SO	**2699.2**	**0**
A + *SI* + SO + *SI:SO*	**2700.6**	**1.4**
A + H + SI + SO + SI:SO	2701.6	2.4
A + H + SI + SO + A:H + SI:SO	2702.2	3.0
A + H + SI + SO + SL + A:H + SI:SO	2703.8	4.6
A + H + SI + SO + SL + A:H + H:SL + SI:SO	2705.7	6.5
A + G + H + SI + SO + SL + A:H + H:SL + SI:SO	2709.1	9.9
A + G + H + SI + SO + SL + A:H + H:SI + H:SL + SI:SO	2713.3	14.1
A + G + H + SI + SO + SL + A:H + A:SI + H:SI + H:SL + SI:SO	2717.1	17.9
A + G + H + SI + SO + SL + A:H + A:SI + A:SO + H:SI + H:SL + SI:SO	2722.5	23.3
A + G + H + SI + SO + SL + A:H + A:SI + A:SO + H:SI + H:SL + SI:SO + A:H:SO	2730.2	31.0
A + G + H + SI + SO + SL + A:H + A:SI + A:SO + H:SI + H:SL + G:SO + SI:SO + A:H:SO	2739.4	40.2

FIGURE LEGENDS

Figure 31. Maps of Barro Colorado Island with ARCGIS forest property overlays. Red lines represent the 27 trails on BCI that were scanned every 30 paces. Symbols on the trail lines represent the number of *A. trigona* nests observed at each location. *Circles* = nests >100 m long, *squares* = nests 50-100 m long, and *triangles* = all nests < 50 m. a) Geological formations, b) Common soil types, c) Forest age, d) Slope degree, and e) Canopy height (m).

Figure 32. Liana removal plots. a) The Gigante peninsula south of Barro Colorado Island, b) Gigante peninsula trail map (white lines) with the sixteen liana removal plots (boxes: *R* = liana removal plots and *C* = control plots), and c) the sixteen survey points layout within each plot.

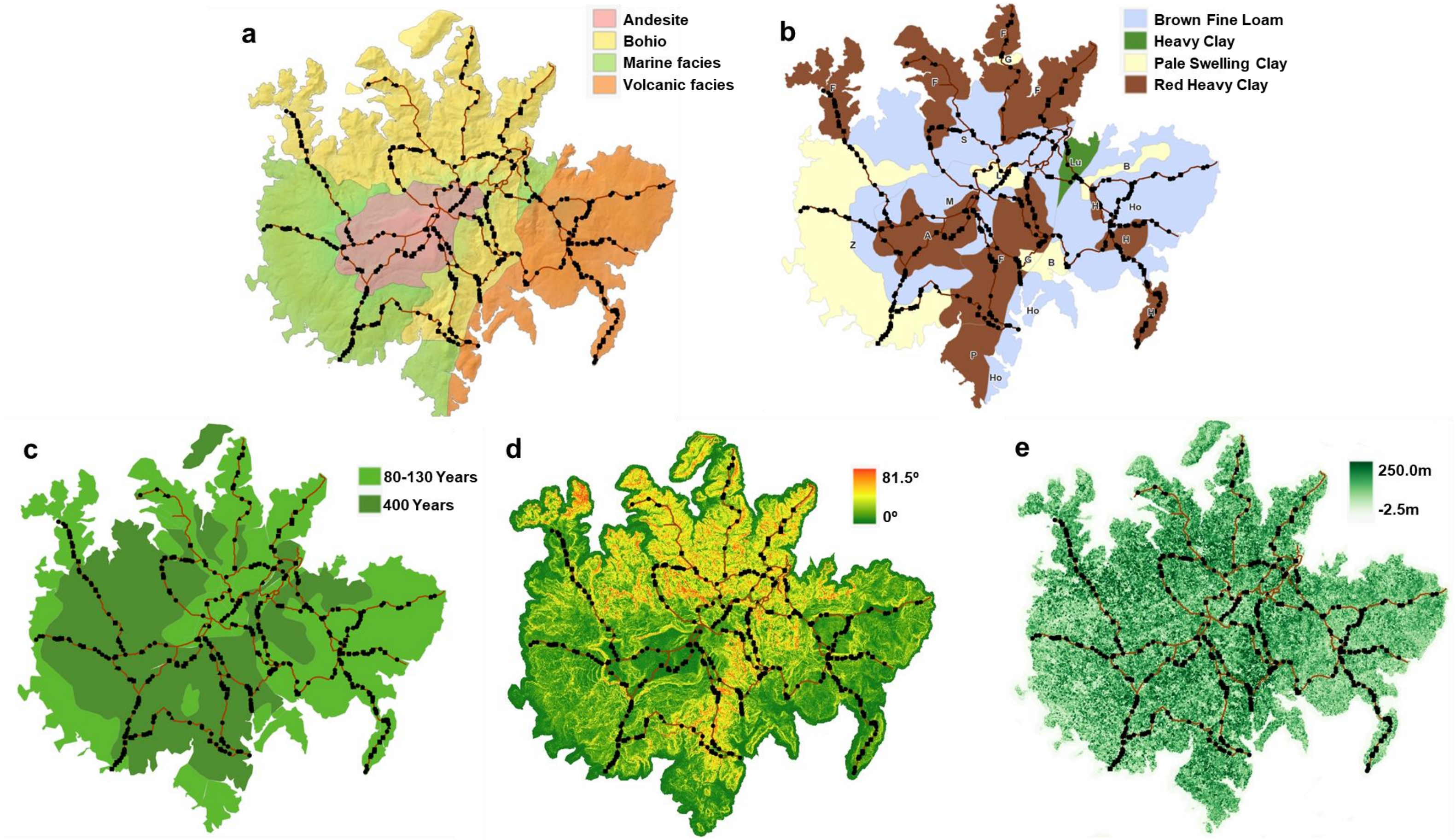
Figure 33. *A. trigona* nests observed at 1,016 survey points on BCI. a) The mean (±SE) nest density among the four geological formations and b) soil types. c) The interaction of forest age (open vs closed circles) and canopy height on the density of *A. trigona* nests with glm regression lines grouped by forest age.

Figure 34. The mean (±SE) density of *A. trigona* nests based on size within forested areas with different soil types. *Circle* = nests < 25 cm, *Squares* = nests 25-50 cm, *Triangles* = nests 50-100 cm, and *Diamonds* = nests > 100 cm.

Figure 35. The number of *A. trigona* nests in 8 liana removal and 8 control plots pre-liana removal (2010) and each year after for nine years.

Figure 36. The mean (±SE) number of nests observed between the liana removal and control plots eight years after liana removal. Symbols represent length of nests: *Circles* = <25 cm, *Squares* = 25-50 cm, *Triangles* = 50-100 cm, and *Diamonds* = >100 cm.

Figure 31.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Andesite
Bohio
Marine facies
Volcanic facies
b
Brown Fine Loam
Heavy Clay
Pale Swelling Clay
Red Heavy Clay
F
G
F
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c
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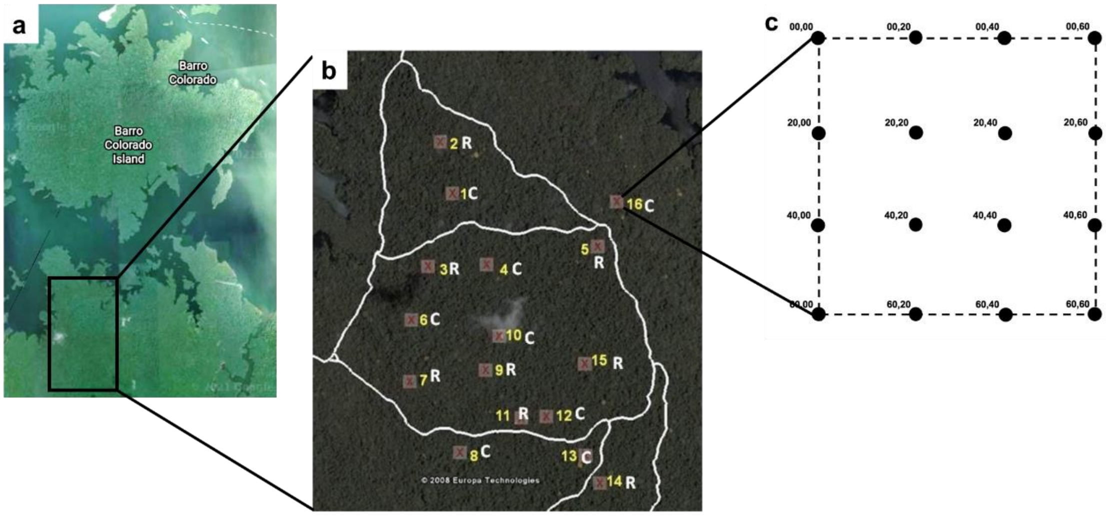
e
250.0m
-2.5m

Figure 32.


a
Barro Colorado
Barro Colorado Island
b
2 R
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5 R
3R
4 C
6C
10C
15 R
9R
7R
11 R
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Figure 33.

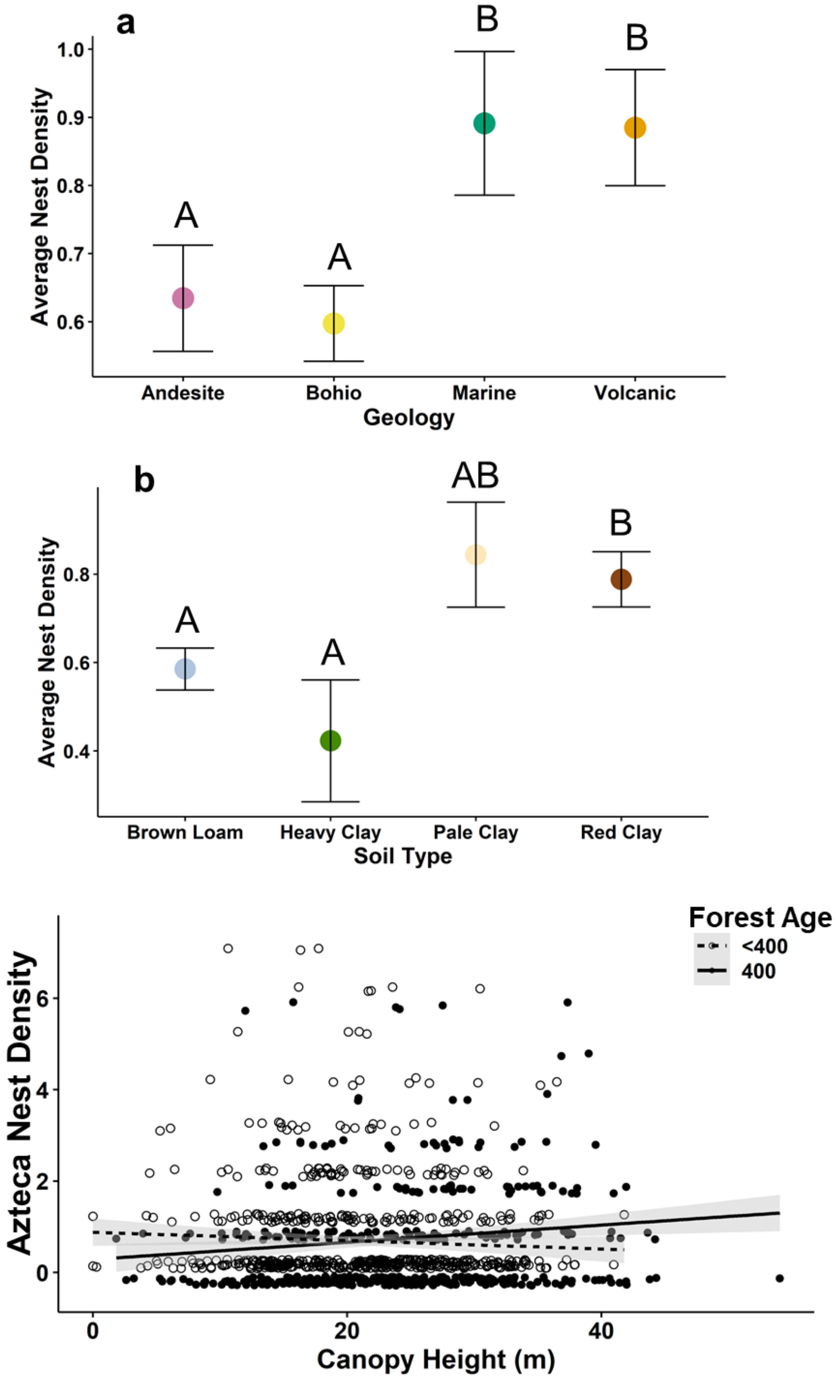
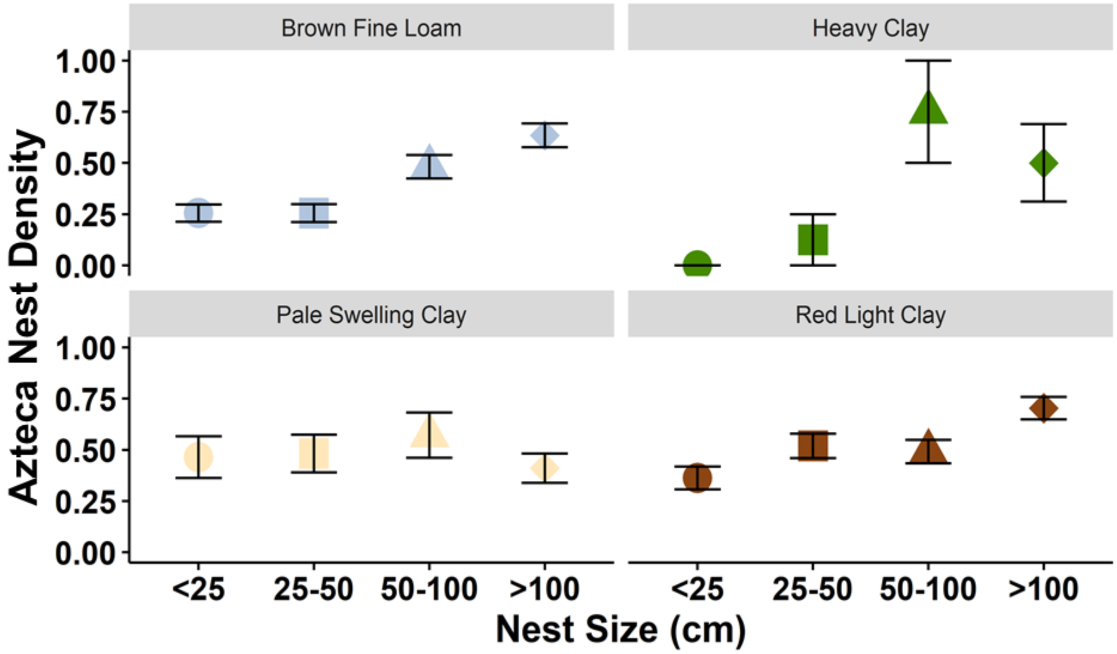

a
Average Nest Density
1.0
0.9
0.8
0.7
0.6
A
A
B
B
Andesite
Bohio
Marine
Volcanic
Geology
b
Average Nest Density
0.8
0.6
0.4
A
A
AB
B
Brown Loam
Heavy Clay
Pale Clay
Red Clay
Soil Type
Forest Age
<400
400
Azteca Nest Density
6
4
2
0
0
20
40
Canopy Height (m)

Figure 34.



Brown Fine Loam
Heavy Clay
Pale Swelling Clay
Red Light Clay
Azteca Nest Density
1.00
0.75
0.50
0.25
0.00
<25
25-50
50-100
>100
Nest Size (cm)

Figure 35.

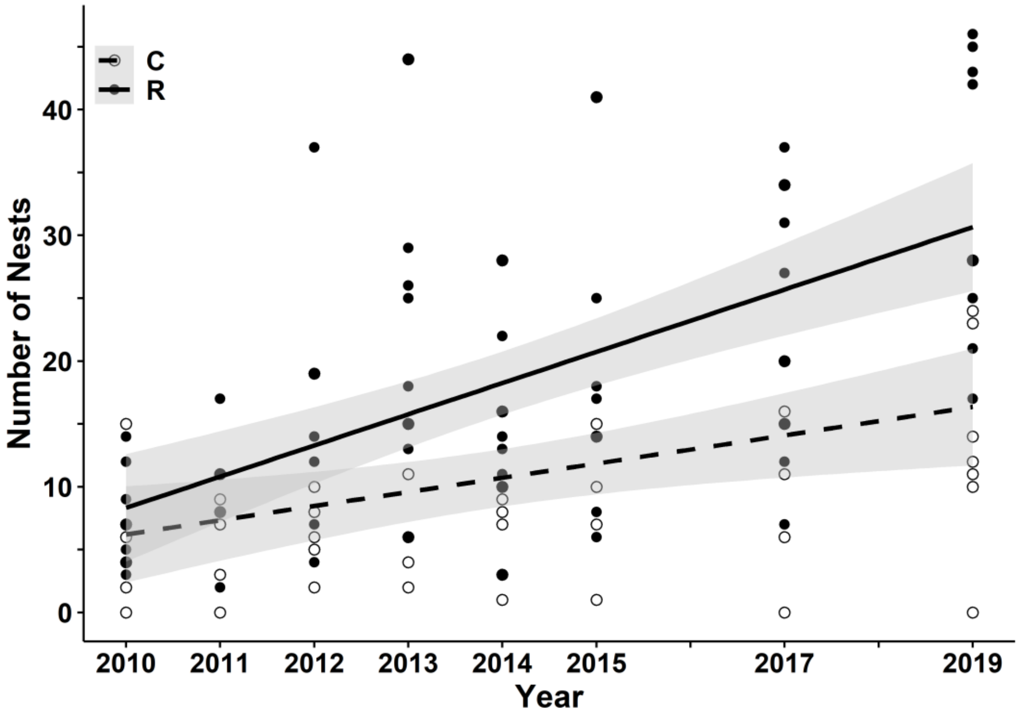
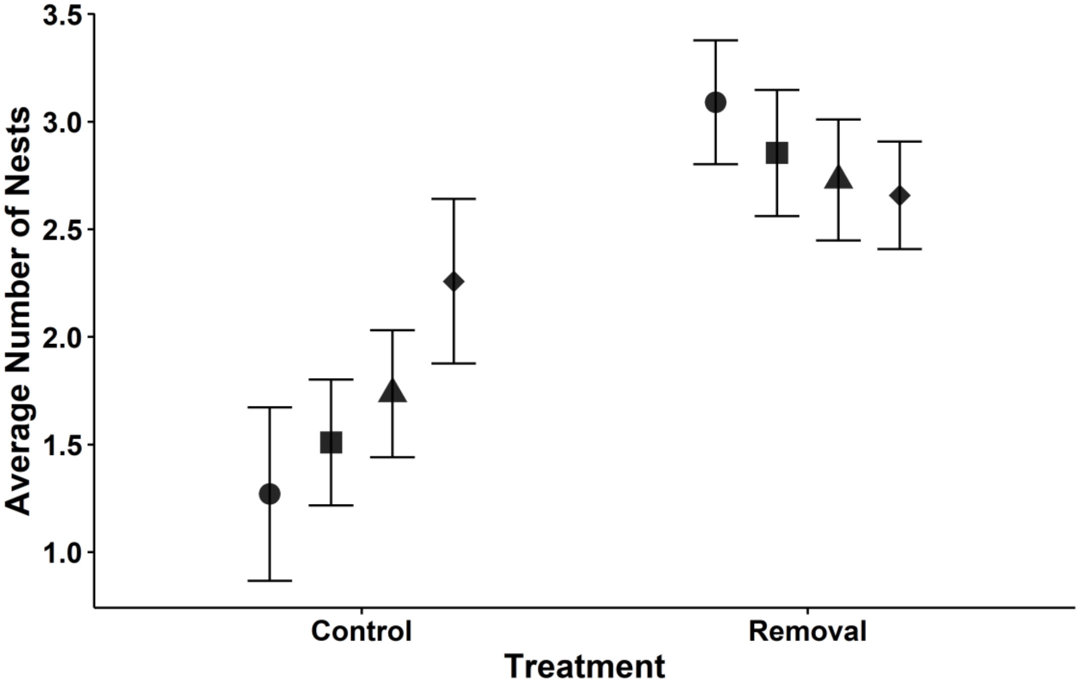


Figure 36.



CHAPTER VI

SUMMARY AND FUTURE DIRECTIONS

Azteca trigona ants (Formicidae:Dolichoderinae) are a major component of the tropical rainforest canopy, dominating the arboreal arthropod community in biomass and via aggressive behaviors. Although *A. trigona* is considered to be a "keystone" species that determines arboreal ant community structure, evidence for this influence is rarely found within non-disturbed habitats. By examining aspects of the behavioral, chemical, and community ecology of this species, I show that only at highly local scales do *A. trigona* affect the behaviors and distributions of select species within a structurally complex forest. The results of these studies indicate that the influence of *A. trigona* ants on heterospecific ant worker behaviors and distributions is less important to canopy ant communities than otherwise presumed and provides a future framework for assessing the impact of dominant species.

Azteca ants have a highly developed chemical defense system, although the ecological impacts of this system are rarely studied. I determined that multiple heterospecific ant species use *A. trigona* alarm pheromones to direct their behaviors, likely to avoid aggressive interactions with *A. trigona* workers. Furthermore, *A. trigona* worker volatiles are not similar to the volatiles produced by eavesdroppers, suggesting that interspecific responses to *A. trigona* are not due to a shared composition of volatile odors. Such eavesdropping presumably is selectively advantageous for eavesdroppers and may influence the distributions and foraging patterns of ants in tree crowns with *A. trigona* colonies. When *A. trigona* nests are disturbed, workers increase their defensive behaviors and the compounds associated with *A. trigona* alarm pheromones (i.e., 2-heptanone and sulcatone) become much more concentrated in the air space surrounding *A. trigona* nests. Disturbed *A. trigona* are likely a distinguishing source of information to eavesdropping competitors or natural enemies. However, disturbed *A. trigona* nests (i.e., nests with physical signs of damage) are rarely observed on BCI and the air space surrounding undisturbed nests does not differ from ambient forest odors. Thus, *A. trigona* nests are potentially rarely chemically distinguishable from the surrounding forest and such chemical camouflage presumably is advantageous for *A. trigona* colonies. Specifically, the potential cost of eavesdroppers could be a strong selective pressure that constrains the "leakage" of reliable, vulnerable signals. Species that do eavesdrop on *A. trigona* pheromones are likely to encounter *A. trigona* regularly in the canopy, by occupying similar resources within or nearby *A. trigona* colonies.

At the level of an individual tree crown, *A. trigona* presence did not affect the frequency, species richness, or composition of artificial nest occupants in a tree. However, at a relatively finer scale (i.e., among different nest substrates and within *A. trigona* foraging ranges), this dominant species affected the frequency of artificial nest occupancy and the composition of nesting species. Such patterns suggest that any negative pressures exerted by *A. trigona* are exclusive within *A. trigona* territorial boundaries, and these effects may be inconsequential to the majority of cooccurring species. When lianas are removed from the forest canopy, the number of *A. trigona* nests remain similar to control areas. However, there are more smaller nests in removal versus control plots and similar patterns were observed within areas of BCI associated with greater tree fall disturbance. Additionally; *A. trigona* nests are more frequently located on taller trees within older forests. The interplay between spatial heterogeneity and resource availability is thus an important component that affects the density and influence of this common species.

Collectively, the results of this dissertation advance our understanding of the effects of a behaviorally dominant arboreal ant on species interactions and access to canopy resources. In particular, this research suggests that canopy ant interactions are affected by a variety of abiotic and biotic factors, that each may influence species in different ways. Thus, this work lays a framework for future experimental research to test which combination of factors are most important for canopy ant communities. Specifically, future research should address the interplay of dominant species effects on the number and type of available resources in diverse types of habitats (such as agroforestry, secondary forests, and primary forests). Additionally, long-term studies of these interactions are needed to further address if observed patterns are real.

Ants are a primary component of the tropical forest canopies and outcomes of species interactions can shape the behaviors and distributions of heterospecific species. Behaviorally and numerically dominant species are expected to exert greater negative pressures on interspecific species, although such pressures are likely mitigated with greater resource availability and eavesdropping interactions. Understanding the interplay of dominant pressures on eavesdroppers, access to resources, and resource availability is fundamental to the understanding of arboreal ant patterns of diversity.

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APPENDIX I:

CHAPTER I LITERATURE REVIEW

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78. Corbara, B. et al. Diversity and nest site selection of social wasps along Guianese forest edges: assessing the influence of arboreal ants. C. R. Biol. 332, 470–479 (2009).

79. Mathis, K. A., Philpott, S. M. & Moreira, R. F. Parasite lost: Chemical and visual cues used by *Pseudacteon* in search of *Azteca instabilis*. J. Insect Behav. 24, 186–199 (2011).

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81. Do Nascimento, R., Billen, J., Sant'Ana, A., Morgan, E. D. & Harada, a Y. Pygidial gland of *Azteca* NR *bicolor* and *Azteca charifex*: Morphology and chemical identification of volatile components. J. Chem. Ecol. 24, 1629–1637 (1998).

82. McCann, S. et al. Strike fast, strike hard: The red-throated caracara exploits absconding behavior of social wasps during nest predation. PLoS One 8, e84114 (2013).

83. Mathis, K. A. & Tsutsui, N. D. Dead ant walking: A myrmecophilous beetle predator uses parasitoid host location cues to selectively prey on parasitized ants. Proc. R. Soc. B Biol. Sci. 283, 20161281 (2016).

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86. Mathis, K. A. & Tsutsui, N. D. Cuticular hydrocarbon cues are used for host acceptance by Pseudacteon spp. phorid flies that attack *Azteca sericeasur* ants. J. Chem. Ecol. 42, 286–293 (2016).

87. Choe, D. H., Villafuerte, D. B. & Tsutsui, N. D. Trail pheromone of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae). PLoS One 7, e45016 (2012).

CURRICULUM VITAE

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Ecology, Evolution, and Behavioral Biology	Office:335
University of Louisville	(918)774-8175
Louisville, Kentucky	rlwell07@louisville.edu

EDUCATION

Present	Ph.D., University of Louisville, Department of Biology
2018	Ant Course, California Academy of Science, French Guiana: Nouragues Field Station
2017	Ants of the Southwest, American Museum of Natural History, Southwest Research Station
2016	B.A., Hendrix College, Double Major in Biology and German Language

PUBLICATIONS

Adams, R.M.M., **Wells, R.L.,** Yanoviak, S.P., Frost, C.J, and E.G.P. Fox. 2020. Interspecific eavesdropping on ant chemical communication. Frontiers in Ecology and Evolution 8: 24.

Wells, R.L., Murphy, S.K., and M.D. Moran. 2017. Habitat modification by the leaf-cutter ant, *Atta cephalotes*, and patterns of leaf-litter arthropod communities. Environmental Entomology 46: 1264-1274.

Moran, M.D., Cox, A.B., **Wells, R.L.**, Benichou, C.C., and M.R. McClung. 2015. Habitat loss and modification due to gas development in the Fayetteville Shale. Environmental Management 55: 1276-1284.

INVITED PRESENTATIONS

Wells, R.L. Dominant Ants: Do they really run the (canopy) world? Organization for Tropical Studies Seminar, Barro Colorado Island 2019.

Wells, R.L., Frost, C.J., and S.P. Yanoviak. Chemical and behavioral influence of *Azteca trigona* on BCI. Bambi Seminar Series, Barro Colorado Island 2018.

Wells, R.L., Frost, C.J., and S.P. Yanoviak. The influence of *Azteca trigona* on forest odors and canopy ant behaviors. Ant Course 2018, Nouragues Field Station, French Guiana.

Wells, R.L., Frost, C.J., and S.P. Yanoviak. The influence of an aggressive and odorous ant on the canopy ecosystem. Smithsonian Tropical Research Institute Tour Guide Conference 2018, Barro Colorado Island, Panama.

Wells, R.L., Cox, A.B., Benichou C.C., McClung, M.R., and M.D. Moran. 2014. Land use change in north central Arkansas due to the Fayetteville shale development. Hendrix Biological Society Meeting, Conway, Arkansas.

PRESENTATIONS

Wells, R.L. and S.P. Yanoviak. 2020. Effects of a common tropical ant on canopy ant access to resources. UofL's Graduate Student Regional Research Conference, Louisville, Kentucky.

Wells, R.L. and S.P. Yanoviak. 2019. How a dominant Neotropical ant influences access to nest resources. Simposio de Mirmecologia, Belo Horizonte, Brazil.

Wells, R.L. and S.P. Yanoviak. 2019. Influence of the Neotropical ant, *Azteca trigona*, on arboreal ant access to nest resources. Ecological Society of America, Louisville, Kentucky.

Wells, R.L., Frost, C.J., and S.P. Yanoviak. 2018. The influence of a tropical canopy ant on forest odor composition and heterospecific ant behaviors. Entomological Society of America, Vancouver, British Columbia.

Wells, R.L., Frost, C.J., and S.P. Yanoviak. 2018. The effects of *Azteca trigona* pheromones on forest odor composition and heterospecific ant behaviors. International Union for the Society of Social Insects, Guaruja, Brazil. **First Place Poster Presentation.**

Wells, R.L., Frost, C.J., and S.P. Yanoviak. 2017. Environmental effects on the composition of *Azteca trigona* nest odor plumes. Entomological Society of America, Denver, Colorado. **First Place: Graduate SysEB Hymenoptera III Award.**

Wells, R.L., Frost, C.J., and S.P. Yanoviak. 2017. Characterizing *Azteca trigona* ant odor plumes in a tropical forest. Midwest Ecology and Evolution Conference, Champaign, Illinois.

Wells, R.L., Frost, C.J., and S.P. Yanoviak. 2016. Chemical characterization of odor plumes around nests of the tropical ant *Azteca trigona*. Kentucky Academy of Science, Louisville, Kentucky. **Third Place: Graduate Ecology and Evolution Science Award.**

Wells, R.L., S. K. Murphy, and M.D. Moran. 2016. The role of the leaf-cutter ant (*Atta cephalotes*) in structuring leaf-litter arthropod communities. Ecological Society of America, Fort Lauderdale, Florida.

Wells, R.L., S. K. Murphy, and M.D. Moran. 2016. Indirect effects of leaf-cutter ants on the litter arthropod community in a tropical rainforest. Arkansas Academy of Science , Fayetteville, Arkansas. **First Place: Undergraduate Field, Organism, and Ecology Award.**

Wells, R.L., Bidny, D., Ellwanger, C., and Fant, J.B. 2015. *Platanthera leucophaea*: an assessment of genetic diversity in relation to pollinator presence and morphological fitness in the federally threatened Eastern Prairie Fringed Orchid. Chicago Botanic Garden Research Symposium, Glencoe, Illinois.

MEDIA ATTENTION

"Hunting for an elusive orchid" – *Open Spaces* – February 2016

"Chicago Botanic Garden and Service interns use genetic clues to guide imperiled orchid restoration efforts" – *US Fish and Wildlife Service Field Notes* – August 2015

GRANTS AND AWARDS

Dissertation Completion Grant, University of Louisville – 2021	$5,500
Graduate Research Fellow, National Science Foundation –2018-2021	$138,000
University Fellowship, University of Louisville – 2016-2020	$73,000
EVPRI Research Grant, University of Louisville – 2018 C.J. Frost and S.P. Yanoviak – Participated in writing grant	$10,000
IUSSI Research Conference, 1st Place Poster Presentation – 2018	$1,000
Biology Graduate Research Grant, University of Louisville – 2018	$175
Travel Fund Endowment, University of Louisville Biology – 2018	$120
Entomological Society of America, 1st Place Section Presentation – 2017	$75
Odyssey Travel Grant, Hendrix College – 2016	$1,000
Odyssey Undergraduate Research Grant, Hendrix College, 2015	$3,640
REU Internship, Chicago Botanic Garden – 2015	$5,000

MENTORSHIP

Marisa Mathison, Barro Colorado Island, Panama 2019
Field assistant determining ant behaviors in response to disturbances

Kane Lawhorn, Barro Colorado Island, Panama 2018
Field assistant collecting odors of Azteca trigona *ant nests*

Jasmine DiProspero, University of Louisville 2018
Assisted with data analysis of chemical mimicry between tropical ants

Aspen Workman, Barro Colorado Island, Panama 2017
Assisted with running behavioral studies of tropical ants

Noah Gripshover, Barro Colorado Island, Panama 2017
Assisted with running behavioral trials and collecting odor samples of tropical ants

Jordan Duncan, Chicago Botanic Garden's College First Program 2015
Assisted with DNA extractions for the Eastern Prairie Fringed Orchid

TEACHING EXPERIENCE

Adjunct Professor

Animal Diversity Lab, Bellarmine University (Spring 2021)

Graduate Teaching Assistant

Tropical Rainforest Biology, University of Louisville (Summer 2018)
Principles of Quantitative Biology, University of Louisville (Fall 2017 – Spring 2018)
Introduction to Biological Systems, University of Louisville (Fall 2016 – Spring 2017)

Guest Lecturer

Diversity Measures and *Biodiversity and Ecosystem Functioning*, Population and Community Ecology Lectures, University of Louisville (Spring 2019)
Island Biogeography Theory, Conservation Biology Lecture, University of Louisville (Spring 2019)
Cooperation, Animal Behavior Lecture, University of Louisville (Fall 2018)
Tales of a smelly ant: How Azteca trigona *influences the tropical canopy system*, Chemical Ecology Lecture, University of Louisville (Spring 2018)
Species Approaches to Conservation Biology, Conservation Biology Lecture, University of Louisville (Fall 2016)
Climate Change, Introduction to Biological Systems co-lecture, University of Louisville (Fall 2016)

Graduate Teaching Assistant Academy Part I (Fall 2017) & Part II (Spring 2019)

Two semester long course that taught undergraduate-level teaching and learning techniques about active learning, motivation, student development theory, learning assessment, and course/class design.

Undergraduate Teaching Assistant

Plants and People for nonmajors, Hendrix College (Spring 2016)
Cell Biology, Hendrix College (Spring 2015)

Non-biological Experience

Blackbird Academy of the Arts taught classical ballet to students ages 6-15, Conway, Arkansas (Fall of 2014 – Spring of 2016)

VOLUNTEER AND OUTREACH

2021 **BIOL 241/243 CURE, University of Louisville**
Member of the team designing laboratory curriculum that includes undergraduate research experiences for the introductory biology labs at the University of Louisville

2016-2021 **Biology Graduate Student Association, University of Louisville**
Active member (2016-2021); Graduate Student Committee Representative (2018-2020); Secretary (2018); Undergraduate Representative (2017)

2018-2020 **Graduate Student Council, University of Louisville**
Director of Professional Development (2019-2020); Biology Department Representative (2018-2020)

2019, 2020 **Day of Science, University of Louisville**
Worked with 45 middle schoolers to teach about tropical insects and climate change

2019 **Louisville Regional Science and Engineering Fair, Louisville, KY**
Judged the Animal Science middle school students

2018 **Bambi Seminar Series, Barro Colorado Island, Panama**
Bambi Jefe: Planned the research talks for scientists working for or with the Smithsonian Tropical Research Institute

2017 **Research and Mentorship Fair, University of Louisville**
Biology department representative

2017 **Capybara Seminar Series, Barro Colorado Island, Panama**
Organized Barro Colorado Island resident research talks

2016 **Kentucky Academy of Science Annual Conference**
Assisted with set-up and coordination of the 102nd annual meeting

2016 **Bioblitz, Lincoln Memorial National Monument, Indiana**
Assisted in insect collections and identification

UNDERGRADUATE RESEARCH EXPERIENCE

2015-2016 **Hendrix College, Department of Biology (Self-designed project)**
Determined the indirect effects that leafcutter ant nests have on arthropod communities in Costa Rican rainforests in a self-designed and fully-funded research project

2015	**Chicago Botanic Garden, Plant Biology and Conservation (C Ellwanger and JB Fant)** *Assessed the genetic diversity of the federally threatened Eastern Prairie Fringed Orchid,* Platanthera leucophaea*, during a 10-week National Science Foundation Research Experience for Undergraduates internship*
2013-2014	**Hendrix College, Department of Biology (MR McClung and MD Moran)** *Worked with Google Earth and Microsoft Excel in an effort to determine the land use change from the natural gas development in the Arkansas shale area*