

Collective Personality in the *Azteca-Cecropia* Mutualism

by

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A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2018 by the
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May 2018

ABSTRACT

For interspecific mutualisms, the behavior of one partner can influence the fitness of the other, especially in the case of symbiotic mutualisms where partners live in close physical association for much of their lives. Behavioral effects on fitness may be particularly important if either species in these long-term relationships displays personality. Animal personality is defined as repeatable individual differences in behavior, and how correlations among these consistent traits are structured is termed behavioral syndromes. Animal personality has been broadly documented across the animal kingdom but is poorly understood in the context of mutualisms. My dissertation focuses on the structure, causes, and consequences of collective personality in *Azteca constructor* colonies that live in *Cecropia* trees, one of the most successful and prominent mutualisms of the neotropics. These pioneer plants provide hollow internodes for nesting and nutrient-rich food bodies; in return, the ants provide protection from herbivores and encroaching vines. I first explored the structure of the behavioral syndrome by testing the consistency and correlation of colony-level behavioral traits under natural conditions in the field. Traits were both consistent within colonies and correlated among colonies revealing a behavioral syndrome along a docile-aggressive axis. Host plants of more active, aggressive colonies had less leaf damage, suggesting a link between a colony personality and host plant health. I then studied how aspects of colony sociometry are intertwined with their host plants by assessing the relationship among plant growth, colony growth, colony structure, ant morphology, and colony personality. Colony personality was independent of host plant measures like tree size, age, volume. Finally, I tested how colony personality influenced by soil nutrients by assessing personality in the

field and transferring colonies to plants the greenhouse under different soil nutrient treatments. Personality was correlated with soil nutrients in the field but was not influenced by soil nutrient treatment in the greenhouse. This suggests that soil nutrients interact with other factors in the environment to structure personality. This dissertation demonstrates that colony personality is an ecologically relevant phenomenon and an important consideration for mutualism dynamics.

I dedicate this work to my parents, Susan and Andrew Marting, who not only cultivated and nurtured my passion for nature but proved to be quite capable field assistants in times of need.

ACKNOWLEDGMENTS

My advisor, Stephen Pratt, has shown me with unwavering, brilliant support throughout my 7-year graduate career. He struck the perfect balance between providing me with clear guidance and permitting independence. His advice was always direct, clear, and insightful. After every meeting, I felt as if the world made a little more sense. He also had incredible patience with me as I pursued many interest, research and otherwise. I could not have asked for a better advisor, and I will always have the utmost respect and admiration for you, Stephen.

Bill Wcislo, my STRI mentor, had an immense impact on my dissertation by allowing for entire afternoons of meandering discussion on natural history, experimental design, and wild speculation. My committee members, Jennifer Fewell, Bert Hoelldobler, and Juergen Gadau, provided excellent support and advice, helping me to tighten up my ideas and experiments. Thank you for your service.

My field assistants in Panama, Taylor Mazzacavallo and Coline Muller, were incredibly hard working, patient, and resilient in the face of a thousand *Azteca* bites. Thank you for your grit. William Jacome, taught me how to grow plants, and did an amazing job of taking care of my greenhouse when I was not in Panama. Thanks to all from the Gamboa community who helped with data collection in the field and the greenhouse: Gabe Patterson, Andrew Quitmeyer, Danielle Hoogendijk, Maggie Raboin, Susanne Wiesner, Ummat Somjee, Megan Oconnell, Kara Fikrig, Hannah Bregulla, Jullia Legeli, Stephen Orr, Hana Duckworth, Ted Carstensen, Evan Walton, Sebastian Stockmaier, Agustin Diaz, Megan Pendred, Eloïse Lebrun, Yussef Castillo, May Dixon,

Claire Hemingway, Krzysztof Kozak, Aaron Goodman, Eva Gril, Clément Aubert, Lynette Strickland, Barrett Klein, and Brendan Dula. I was overwhelmed with your support, and together, we pulled it off. Any time you need a helping hand, I'll be there.

I would like to express deep gratitude for the undergraduate researchers who slogged through hours of video footage, scoring ant behavior, measuring morphology, and helping with genetic analysis. Thank you, Brandon Aki-Jackson, Moira McCarthy, Danielle Fry, Pat McCarthy, Nan Qui, Zakaria Mahmoud, Nicole Kallman, Melissa Lopez, Vanessa Howg.

The STRI scientists Ben Turner, Klaus Winter, and Allen Herre all provided essential advice when I was designing and setting up experiments. Thank you for your guidance. I thank Raineldo Urriola, the problem solver, and Adriana Bilgray, the organization falcon, for logistical support. Thanks to May Boggess and Irene van Woerden for statistical advice.

Finally, I would like to thank my girlfriend Michelle. She has shown me unconditional support throughout multiple 6-month field seasons and aided me with things I neglected during the final deep writing phase. Thank you for your love and support.

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CHAPTER 1

DISSERTATION INTRODUCTION

The Azteca -Cecropia Mutualism

Two Impressive Genera Unite

Cecropia is one of the most prominent and unique genera of pioneer plants in the neotropics (Figure 1.1). With over 60 species ranging from southern Mexico to northern Argentina (Berg et al. 2005), the plant's unrivaled success is due to a number of specialized adaptations - most notably its intimate symbiosis with *Azteca* ants. Ants in the genus *Azteca* are notoriously aggressive and carnivorous, often dominating the forest canopy (Davidson 2003, Ribeiro et al. 2013). At least 13 species are obligate symbionts of *Cecropia* trees (Longino 2007). The unity of these two genera goes back about 8 million years (Gutiérrez-Valencia et al. 2017) to form one of the most impressive mutualisms in the world.

In the Beginning

A newly mated queen chews a hole at a designated site on a hollow internode of a half-meter *Cecropia* sapling. This site opposite the leaf petiole, called a prostoma looks like a dimple where the plant's wall is shallow and designed for ant excavation (Davidson 2005). Once inside, she scrapes the internal tissue, called parenchyma, and uses it to plug the hole (Valverde and Hanson 2011). In a compartment in her mouth call a buccal

cavity, she carries a special fungus that she has taken from her home colony, which she cultivates inside her new internode (Mayer et al. 2018). She will lay eggs and tend to the larvae, feeding them from the fungus until the first workers emerge and reopen the hole.

When a queen enters a *Cecropia* internode, she may not be the first one there. If she lands on a sapling in which workers have not emerged yet, the queen can enter an internode that is already occupied by queens. Commonly, two to five queens inhabit the same internode and cooperate in raising brood together. Thus, each internode functions as its own cohesive colony after the workers emerge. Every tree potentially contains several multi-queen colonies who compete for dominance over the tree, mostly by excluding other colonies from collecting the plant's food bodies. Patrolling workers also attack and kill new queens that land on the tree looking to start a colony (Perlman 1992).

After one colony reigns supreme and eliminates all other colonies, a period of ergonomic growth follows. Both the winning colony and the tree grow considerably in size over a few months of stockpiling food bodies. At this point, the cooperating queens turn on each other, likely concerned with the production of sexuals, new queens and males, that will fly off and pass their genes on. The queens gruesomely fight to the death, tearing limbs and severing segments, until one queen is victorious, remaining as the sole reproductive for the rest of the colony's life (Figure 1.2, Perlman 1992).

From a queen's perspective, multi-queen colonies are advantageous over single-queen colonies in the first stage of competition because they can quickly produce more workers that help them gain control of the tree. However, when the colony has the resources to produce new queens and males, the genes being passed on may be split

between the remaining queens. Thus, it is advantageous to a queen to be the only reproductive, so she can ensure all offspring are hers (Perlman 1992).

Mutualistic Adaptations

Cecropia plants are pioneers, meaning they are the first to colonize sunny disturbed areas like treefall gaps. Sunlight is a rare resource in the rainforest so many plant species fiercely compete to outgrow one another (Figure 1.3). *Cecropia* is among the fastest of these species, putting out a new leaf-bearing internode at its apex every one to four weeks (depending on the species). The fast-growing internodes are hollow, providing a spacious, temperature-controlled refuge for *Azteca* colonies to nest within the tree (Figure 1.4). Internodes are easily entered through the specialized prostomata (mentioned above) and septa between internodes are often chewed to create a continuous cavity inside the length of the stem (Longino 1991a). The spongy, white parenchyma tissue lining new internodes is nutritious and can also be used to create additional organizational structures within the internodes (Valverde and Hanson 2011).

In addition to a cozy shelter, the plant also serves a nutritious diet to their hard-working colony. At the base of each giant radial leaf where the petiole meets the tree stem, there is a hairy, specialized structure called a trichilium that pumps out Müllerian food bodies (Figure 1.5). These beautiful white ovoids, just bigger than an ant egg, perfectly fit between the workers' mandibles and are packed with nutritious glycogen (Rickson 1976). For a balanced diet, the plant also provides translucent, fat-rich pearl bodies at sites across the underside of the leaves (Rickson 1976).

In return for food and shelter, the colony acts as an effective defense system for their tree and significantly reduces herbivore leaf damage and competing vines (Janzen 1969, Schupp 1986). Workers actively patrol the tree's stem and leaves 24 hours a day, guarding it against intruders. If they encounter an insect herbivore, they will quickly attack it with their sharp mandibles and lift their gaster to spray a chemical spray composed of iridoids (Dejean et al. 2009). They often release an alarm pheromone that recruits nearby patrollers to the threat. Often, the intruder quickly retreats and escapes. However, specialized hairs on the surface of the plant work together with hooks on the ants' legs that increases grip strength, allowing them to anchor large prey like katydids or grasshoppers (Dejean et al. 2010). Occasionally, the workers are able to immobilize and kill the intruder, chopping it into small pieces and storing them inside the tree for later consumption (Figure 1.6). Workers will also fiercely attack much larger herbivores like sloths (Appendix A) and monkeys (Appendix B). They will climb on top of the intruder, boarding it by the hundreds in search of a soft, sensitive spot where they can sink their mandibles in.

Workers even respond to the tree's distress cues. When a patroller encounters fresh leaf damage, volatile chemicals released by the plant alert the ant that there was recent danger (Agrawal and Dubin-Thaler 1999). The ant responds by doing a brief, agitated sweep search before dotting a chemical trail from the leaf, down the petiole, and into the nearest stem entrance. A pulse of workers file out, following the trail to the specific site of the damage to search for the culprit.

In addition to being fierce warriors, *Azteca* are also diligent house keepers and gardeners. They clear dirt and bits of debris off the leaf surfaces which ensures maximum

photosynthetic potential. Also, fast-growing vines often plague pioneer species and compete for their sunlight. When foreign plant material contacts the *Cecropia* tree, patrollers distinguish it from their host and attack it. Workers chew on the meristem until it dies and the vine falls from the tree (Figure 1.7, Janzen 1969). The colony can also fertilize its host. Nitrogen consumed by the ants is passed to the plant and can be found in its leaf tissue (Sagers et al. 2000, Dejean et al. 2012). Colonies maintain refuse piles inside the internodes and it is likely the nutrients are absorbed there, but the mechanism is still unknown.

One reason ants destroy vines is to eliminate points of entry that can be used by intruders (Davidson et al. 1988). For a well-maintained tree, an intruder will only have access to the plant where the central stem meets the ground. If a tree falls in a storm or a landslide, many intruders try to take advantage of the colony's structural vulnerability.

The Symbiotic Ecosystem

Once you dissect open a *Cecropia* tree and expose its internal compartments, you'll immediately notice that ants and plants are not the only players in this story. There is a complex web of organisms whose intricacies and interactions are still largely unknown (Figure 1.8).

One of the first things you may notice are the flat ovals that cling to the wall. These mealybugs and scale insects have straw-like mouth parts to suck sap from the phloem of the tree (Longino 1991a). Because sap has such a high carb-protein ratio, the insects need to consume a lot to get enough protein for a balanced diet. They secrete the

unused carbohydrates as a rich, sugary liquid that the *Azteca* ants love. Ants protect and tend scale insects like cattle inside the internodes of the tree, making sure they have good feeding sites and culling the population to prevent plant-damaging outbreaks.

Another observation you may make is that the colony's brood are often placed around a chocolate-colored, bulbous dome. Upon inspection with a dissection scope, you can see this pile is host to a sea of writhing nematodes. Little is known about their function, but they have mouthparts consistent with bacteria-feeding nematodes (Esquivel et al. 2012). It's possible they may provide a hygienic service for the brood, but more experiments are needed.

Microbial communities play a big role in the nests of many ant species (such as leafcutters), and this is likely also true in the *Azteca* - *Cecropia* system. Bacterial communities differ in richness and abundance in certain internodes and between different areas of the plant. A certain group of fungus called Chaetothyriales is abundant inside *Cecropia* trees, especially residing in the nematode and refuse piles (Nepel et al. 2016). The fungal species that make up these communities depend on the *Azteca* species rather than the *Cecropia* species. Currently, the functional significance of these communities is unknown, but the possibilities are endless - ranging from parasitic to mediating nutrient transfer between the colony and the tree.

Phorid flies are attracted to the *Azteca* alarm pheromone and lay eggs on distracted workers (Mathis et al. 2011). Their maggots can be found inside the internodes, rummaging through the colony refuse piles.

The specific relationships between these groups have hardly been studied. Many other unknown organisms that await discovery are undoubtedly interacting inside of this symbiotic ecosystem.

Animal Personality and Behavioral Syndromes

A Widespread, Important Phenomenon

A central goal of behavioral ecology is to understand the causes and consequences of consistent individual differences in behavior (Sih et al. 2004b, 2010, Réale et al. 2007, 2010, Sih and Bell 2008, Dingemanse et al. 2010, MacKay and Haskell 2015, Bengtson et al. 2018, Dammhahn et al. 2018). Individuals in a wide range of taxa display repeatable variation in behavioral traits that is structured in a meaningful way (Sih et al. 2010). Over the last decade, we have seen significant developments on how behavioral strategies that are stable over time and across contexts are associated with many important aspects of biology, including genetic variation (Fidler et al. 2007), heritability (Drent et al. 2003), developmental conditions (Fairbanks and McGuire 1993), experience (Frost et al. 2007), cognition (Sih and Del Giudice 2012), physiology (Huntingford et al. 2010), state variables (Sih et al. 2015), life-history strategy (Figueredo et al. 2005, Dammhahn et al. 2018), environmental conditions (Pinter-Wollman et al. 2012a), and fitness (Smith and Blumstein 2008)

The structure of variation is most often described with the terms “personality” and “behavioral syndrome.” An animal’s personality refers its reaction style to a set of

stimuli; personalities occur when behavioral traits differ consistently among individuals across time (MacKay and Haskell 2015). A behavioral syndrome describes the relationship among consistent traits across individuals in a population, or suites of consistent behavioral tendencies (Sih et al. 2004a). Within a population that displays a behavioral syndrome, individuals possess different personalities or behavioral types (Sih et al. 2004b). This framework focuses on differences between animals rather than ignore them, with implications for better understanding of the biology of the individual.

Collective Personality

Although behavioral syndromes are now known in individuals for a wide range of taxa (Sih et al. 2010), studies have only recently focused on collective behavioral syndromes emerging from groups of highly social individuals, such as social insect colonies (Chapman et al. 2011, Wray et al. 2011, Pinter-Wollman et al. 2012a, 2012b, Scharf et al. 2012, Modlmeier et al. 2012, 2014a, 2014b, Bengston and Dornhaus 2014, Keiser et al. 2015, Blight et al. 2016). Conceptualizing social insect societies as singular functioning superorganisms can be useful because natural selection acts at multiple levels (Wilson and Sober 1989, Hölldobler and Wilson 2009), which highlights the impact that collective behavior can have on fitness. Studying behavioral syndromes in social insects enables us to test mechanisms controlling behavioral types, because they allow for behavioral characterization and manipulation at different levels of biological organization – both the individual worker and the collective.

Recent studies on colony personality have helped us understand how behavioral traits are structured, and how they relate to physiology, environment, and fitness. I provide a brief overview of notable examples here. Aggression towards enemies in *Temnothorax* ants has been shown to correlate with exploratory behavior both at the worker level and the colony level, and colony aggression was consistent over the course of four to five months (Modlmeier et al. 2012). Colony behavioral type also correlates with physiological traits in *Temnothorax* (Scharf et al. 2012). Tendency to relocate to new nest sites, which exposes colonies to new pathogens, correlates with a colony's immune defense levels. Behavioral type has been shown to affect components of colony fitness. European honey bees maintain a collective behavioral syndrome for defensive response, foraging activity, and undertaking, and these traits correlate with overwintering success (Wray et al. 2011). In the ant *Aphaenogaster senilis*, bold colonies won more intraspecific competitions, but also suffered higher worker mortality in risky foraging trials (Blight et al. 2016). Colony personality correlates with environmental variables. In *Temnothorax* ants, bolder, more aggressive colonies are found at higher latitudes with shorter growing seasons (Bengston and Dornhaus 2014), which likely drives selection for a short, fast-paced life-history strategy (Bengston and Dornhaus 2015). In *Messor* ants, colonies that nested in drier conditions were faster at foraging and responsiveness, spending less time outside to reduce desiccation (Pinter-Wollman et al. 2012a).

To date, most studies test for the presence of a behavioral syndrome under controlled lab conditions, and behavioral types are often then linked to group performance or external factors. However, the range and flexibility of colony traits under controlled, novel environments may vary from that found in nature. With myriad factors

affecting behavior in the wild, inherent behavioral consistency or variation could be masked in laboratory studies, thus limiting their ecological relevance. (Niemelä and Dingemanse 2014). More studies are needed on collective personality in the wild and their interactions with other species and the environment.

Collective Personality in the Azteca-Cecropia Mutualism

The focus of my dissertation is on collective behavioral syndromes in colonies of the neotropical arboreal ant *Azteca constructor*, an obligate inhabitant and mutualist of *Cecropia* trees. *Cecropia* trees are colonized as saplings and the colony develops with its host plant throughout ontogeny (Perlman 1992) and thus colonies are relatively sessile and tied to local environmental factors. *Azteca* ants are an ideal study system because they are abundant in a wide range of habitats throughout the neotropics, have robust, measurable behavior, and have discrete territories and food resources within each host tree (Heil and McKey 2003). Additionally, colonies can easily be studied in the field, in potted plants in the greenhouse, and in synthetic *Cecropia* trees in the lab, making it possible to test a spectrum of questions that balance ecological relevance and environmental control. This system provides a unique perspective on behavioral syndromes through the intimate mutualism with *Cecropia* trees. How might certain behavioral types affect plant health, or conversely, how might plant health effect colony behavioral type? How might colony personality be intertwined with aspects of sociometry and host plant structure? Is colony personality primarily driven by intrinsic

colony factors, or extrinsic, environmental factors? I attempt to answer these questions in the following chapters of this dissertation.

Dissertation Overview

Chapter 2 – I quantified the level of behavioral variation colonies display and how it relates to the health of their mutualist partner (this work is published in Marting et al. 2018). I tested the consistency of five behavioral traits in the field (vibrational disturbance, response to intruder, response to leaf damage, exploratory tendencies, and patrolling behavior), measured colony size, and the total amount of leaf damage for each plant. Collective behavior varied substantially between colonies for all traits measured and four out of the five traits were consistent within colonies and correlated among colonies. This revealed a behavioral syndrome along a docile-aggressive axis, with high-scoring colonies showing greater activity, aggression, and responsiveness. Scores varied substantially between colonies and were independent of colony size and age. Host plants of more active, aggressive colonies had less leaf damage, suggesting a link between a colony's personality and effective defense of its host, though the directionality of this link remains uncertain. We posit three hypotheses regarding the directionality of this relationship; 1) aggressive colonies are better defenders and promote healthier trees, 2) trees with more leaf damage poorly nurture their colonies resulting in less aggressive colonies, or 3) colony aggression and plant health influence each other in a positive feedback loop, stabilizing among-colony differences. This field study shows that colony

personality is an ecologically relevant phenomenon and sheds light on the importance of behavioral differences within mutualism dynamics.

Chapter 3 – For ant colonies that obligately nest within plant hosts, aspects of their sociometry are likely intertwined with their host plant, which has implications for the strength and stability of the mutualism. In the *Azteca-Cecropia* mutualism, tree structure determines where the colony nests in the stem's hollow, segmented internodes. Little is known about how the colony is distributed and organized and how this might affect the colony's ability to respond to threats or opportunities. These ants also express collective personalities that may be influenced by host tree dimensions, colony distribution within the tree, worker body size. I investigated patterns of and relationships among five major categories of sociometry; plant growth, colony growth, colony structure, ant morphology, and colony personality. Colonies and plant grew at the same rate, suggesting a growth feedback loop that stabilizes the mutualism. There was a high level of among-colony variation in vertical nest distribution within the host plant. Colony distribution correlates more strongly with colony size than tree structure, and ant body size correlates more strongly with tree structure than colony size. Colony personality was independent of colony distribution and tree structure but correlated with ant body size such that colonies with smaller, less allometric workers had more aggressive personalities. The results of this study provide insights into how ant-plant structural relationships may contribute to plant protection and the strength of the mutualism.

Chapter 4 – What is driving the differences in personality types? I designed an experiment to test whether collective personality is a fixed, inherent property of the colony or influenced by environmental factors. At the same time, I tested a more specific

hypothesis regarding environmental factors: soil nutrients increase the resources that the plant offers, which in turn increases colony aggression. I conducted a three-phase experiment where I 1) assessed colony behavior in the field, 2) harvested trees, extracted colonies, and transplanted them into greenhouse plants under differing nutrient treatments, and 3) re-assessed colony behavior 10 months later. The results strengthen the evidence that colonies of *Azteca constructor* maintain a collective behavioral syndrome along a docile-aggressive axis under natural field conditions, but we show that the structure of this behavioral syndrome unravels when colonies are transplanted to the greenhouse. Colonies did not behave consistently from the field to the greenhouse, which supports the hypothesis that extrinsic factors control the consistency and correlation of traits likely control the behavioral syndrome in *A. constructor*. Furthermore, soil nutrients correlated with colony personality in the field, but did not influence most colony behaviors in the greenhouse despite substantially increasing resource abundance. Soil nutrients likely interact with other environmental factors to structure their behavioral syndrome in the field.

Chapter 5 – I summarized what we learned about the behavioral ecology of the *Azteca-Cecropia* mutualism. I synthesize results from all chapters to form a working hypothesis about the causes and consequences of the collective behavioral syndrome in the context of this mutualism, highlighting the significant contributions this dissertation makes to the field of animal behavior. Finally, I outline future planned experiments that will further our understanding of this beautiful and fascinating symbiosis.

Figures



Figure 1.1. A photograph of *Cecropia obtusifolia*.

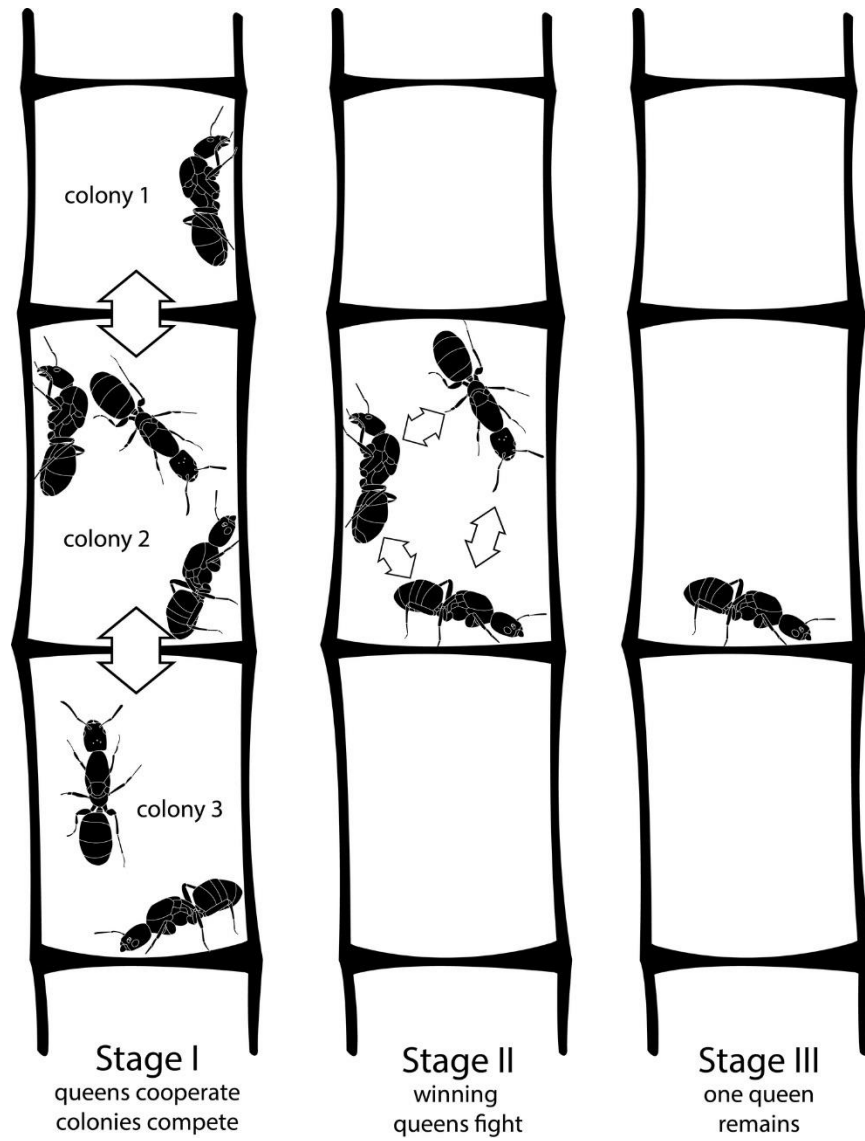


Figure 1.2. A diagram of the stages of colony founding in *Azteca* ants.



Figure 1.3. *Cecropia* seeds lay dormant on the rainforest floor, sometimes for years, awaiting a break in the canopy to spurt a ray of light. The seed then germinates with explosive growth. Soon after, a new *Azteca* queen will colonize the plant, found a colony, and help protect it from other gap-growing competitors. Acrylic on canvas.



Figure 1.4. A queen atop worker-built carton structure inside a *Cecropia* internode.



Figure 1.5. Workers harvesting food bodies.



Figure 1.6. Workers kill and feast on a grasshopper.



Figure 1.7. Workers attacking an encroaching vine.

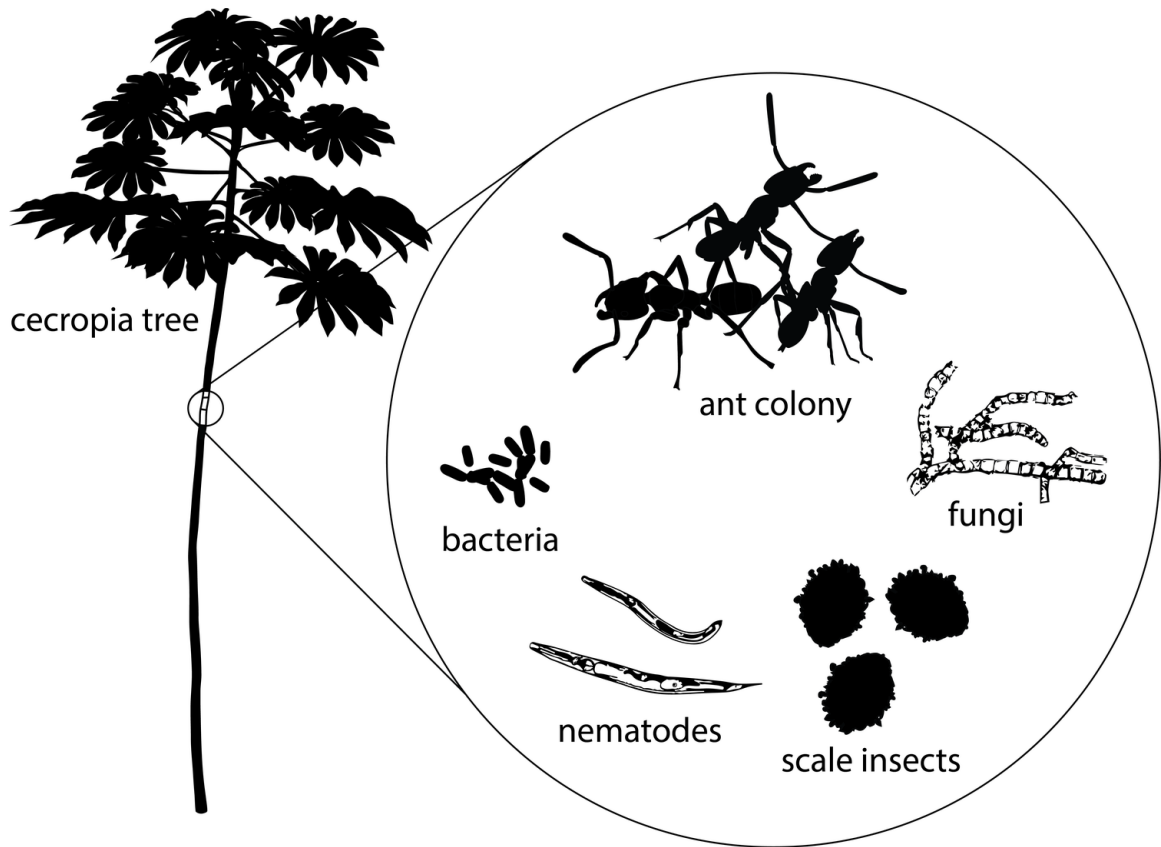


Figure 1.8. A diagram of the organisms involved in the *Azteca-Cecropia* mutualism.

CHAPTER 2
COLONY PERSONALITY AND PLANT HEALTH

(Published in *Behavioral Ecology* 2018, 29:1; see Appendix C for coauthor approval)

Abstract

For interspecific mutualisms, the behavior of one partner can influence the fitness of the other, especially in the case of symbiotic mutualisms where partners live in close physical association for much of their lives. Behavioral effects on fitness may be particularly important if either species in these long-term relationships displays personality. I conducted a field study on collective personality in *Azteca constructor* colonies that live in *Cecropia* trees, one of the most successful and prominent mutualisms of the neotropics. These pioneer plants provide hollow internodes for nesting and nutrient-rich food bodies; in return, the ants provide protection from herbivores and encroaching vines. I tested the consistency and correlation of five colony-level behavioral traits, censused colonies, and measured the amount of leaf damage for each plant. Four of five traits were both consistent within colonies and correlated among colonies. This reveals a behavioral syndrome along a docile-aggressive axis, with higher-scoring colonies showing greater activity, aggression, and responsiveness. Scores varied substantially between colonies and were independent of colony size and age. Host plants of more active, aggressive colonies had less leaf damage, suggesting a link between a colony's personality and effective defense of its host, though the directionality of this link

remains uncertain. This field study shows that colony personality is an ecologically relevant phenomenon and sheds light on the importance of behavioral differences within mutualism dynamics.

Introduction

Behavioral syndromes of individuals are well-known in a wide range of taxa (Sih et al. 2010), and recent studies have further shown collective behavioral syndromes emerging from groups of highly social individuals, such as social insect colonies (Chapman et al. 2011, Wray et al. 2011, Pinter-Wollman et al. 2012a, 2012b, Scharf et al. 2012, Modlmeier et al. 2012, 2014a, 2014b, Bengtson and Dornhaus 2014, Keiser et al. 2015, Blight et al. 2016). Conceptualizing insect societies as singular functioning superorganisms can be useful because natural selection acts at multiple levels (Wilson and Sober 1989, Korb and Heinze 2004, Hölldobler and Wilson 2009), highlighting the impact that collective behavior can have on fitness. Studying behavioral syndromes in social insects enables us to test mechanisms controlling behavioral types, because they allow for behavioral characterization and manipulation at different levels of biological organization – both the individual worker and the colony. With myriad factors affecting behavior in the wild, inherent behavioral consistency or variation could be masked in laboratory studies, thus limiting their ecological relevance (Niemelä and Dingemans 2014). More field-based studies are needed on collective personality and their interactions with other species and the environment.

Ants have an affinity for interspecific mutualisms (Hölldobler and Wilson 1990), which raises interesting questions about how colony personality interacts with the biology of the ants' partners. Well studied mutualisms include species that facultatively tend and protect hemipterans in return for honeydew (Styrsky and Eubanks 2007), fungus-growing ants that obligately farm nutritious fungi (Chapela et al. 1994), and species that protect plants in return for food and/or shelter (Heil and McKey 2003). The existence of differing colony behavioral types has important implications for understanding mutualistic relationships (Sih et al. 2012), and is a poorly explored topic in mutualism ecology (but see Schmiede et al. 2017). Different types may be associated with different life history strategies, which in turn affect the strength of the mutualism. Thus, the behavioral syndrome of a colony can affect the fitness of its partner. In turn, the life history strategy of the colony's partner may influence the behavioral syndrome.

I studied collective behavioral syndromes in colonies of the neotropical arboreal ant *Azteca constructor*, an obligate mutualist with *Cecropia* trees. The symbiosis between *Azteca* ants and *Cecropia* trees is one of the most successful and prominent mutualisms of the neotropics (Davidson and McKey 1993), abundant in disturbed areas and forest gaps ranging from southern Mexico to northern Argentina. These pioneer plants provide ants with hollow internodes as shelter and nutrient-rich food bodies; in return, the ants provide protection from herbivores and encroaching vines (Schupp 1986). The mutualism is a rich system for the study of behavior and ecology because ants have robust, measurable behavior, and discrete territories and resources within each fast-growing host plant (Heil and McKey 2003). These features allow us to investigate interesting questions about the causes and consequences of behavioral variation. The ants' defensive and

exploratory behaviors are of particular relevance to the mutualism. These behaviors include patrolling activity and aggression similar to that seen in non-mutualist ants, but *Azteca* also have specialized behaviors adapted to their symbiosis, such as responding directly to leaf damage (Agrawal and Dubin-Thaler 1999). How these different traits relate to one another is important when assessing behavioral consistency and correlations. In particular, if some colonies have consistently stronger defensive behavior, their host plants potentially suffer less leaf damage and potentially benefit from faster growth rates, which in turn may feed back into colony fitness through growth and reproductive output.

If colonies do show evidence of personality, there are many possible drivers of behavioral difference that must be considered. These include intrinsic factors such as colony age and size, but also environmental factors, such as resource availability, that are intertwined with their *Cecropia* host. Colony nesting space depends on the host tree's internode size, and the ants' nutrition is derived from food body production, which is influenced by light environment (Folgarait and Davidson 1994) and soil nutrients (Folgarait and Davidson 1995). How might canopy cover affect colony behavior? A single *Azteca* species can colonize several *Cecropia* species. Might there be differences in colony behavior depending on the *Cecropia* species they occupy?

I tested the hypothesis that *A. constructor* colonies living in *Cecropia* trees display a collective behavioral syndrome under natural conditions in the field. I measured consistency and correlation in five distinct colony-level behavioral traits related to activity, boldness, exploratory behavior, and response to threats against the ants and their host. I then measured the relationship between colony behavior and host plant leaf damage, canopy cover, *Cecropia* species, colony age, and colony size.

Methods

Study site and colony selection

I located *Azteca constructor* colonies along a 12 km stretch of Pipeline Road in and around the lowland tropical rainforests of Soberania National Park, Colón, Panama between March and May 2013. Based on the correlation between tree height and colony size in related species (Vasconcelos and Casimiro 1997), I limited the variation in colony size by choosing colonies inhabiting similar-sized *Cecropia peltata*, *C. obtusifolia*, and *C. insignis* trees (height \pm SD = 3.43 m \pm 0.83 m, $n = 14$). Trees in these species can reach over 20 m in height and have many branching points, but I used smaller trees with single stems for assay standardization and ease of access. Each tree contained a single colony, which I confirmed by collecting the colony and locating the queen after the behavioral trials. I identified the queens as *Azteca constructor* using keys in Longino (2007). Queen and worker voucher specimens were deposited in the Arizona State University Natural History Collections. I identified the host *Cecropia* species using keys in Berg et al. (2005).

Behavioral traits

To characterize colony-level behavior, colonies were subjected to five bioassays: patrolling behavior, vibrational disturbance, response to intruder, response to leaf

damage, and exploratory tendency (detailed below). Colonies received each assay at least two times to assess behavioral consistency (the patrolling behavior assay was repeated four times per colony). Colonies received only one manipulation per day, and repeated assays were separated by at least 24 hours. *Cecropia* trees have thin, distinct septa lines that segment the stem into discrete internodes. To standardize behavioral measurements across different tree sizes, I focused on the central stem at the lowest leaf's internode (henceforth, the "focal internode"), which I estimated to be the location of median colony distribution based on four preliminary tree dissections. For patrolling behavior, vibrational disturbance, and response to intruder, I scored activity by counting the number of times I saw a worker completely traverse the lower septum line of the focal internode, regardless of direction or ant identity. For leaf damage assays, I focused on an entire leaf instead of the stem and counted the number of workers on that leaf every minute. Trials were recorded with an HD camcorder (Panasonic HC-X900M) between May and August of 2013.

Patrolling behavior – *Azteca* ants constantly patrol stems and leaves of their host plant (Longino 1991a). Patrolling behavior is most closely synonymous with "activity level" measured in similar studies (Bengston and Dornhaus 2014) and can be indicative of a number of colony traits that are not mutually exclusive, including food-body foraging effort and threat detection effort. In addition, the likelihood of a colony detecting and responding quickly to a potential threat increases with the number workers that traverse the stem (Rocha and Bergallo 1992).

To measure patrolling behavior, I counted the absolute worker number, a distinct measure from colony response effort to a given stimulus. I recorded undisturbed

patrolling behavior for five minutes and scored activity as the number of worker crossings of the focal internode.

Vibrational disturbance – *Azteca* are notorious for their aggressive response to vibrational disturbance caused by vertebrate attacks on their host tree (Longino 1991a). Vibrational disturbance is caused when a vertebrate threatens the colony (e.g. anteaters: Hirsch et al. 2014; woodpeckers: Calderon 2011) or the tree (e.g. sloths: Wheeler 1942; monkeys: Silver et al. 1998). Colonies likely require a large response to deter this type of threat because workers do not have stingers and rely on their mandibles to dissuade attacking vertebrates. Deterring vertebrates is probably costly because defending workers that mount the attacker likely die during the attack, though this has not been measured. Therefore, the response to vibrational disturbance indicates colony defensive aggressiveness and risk-taking boldness.

I simulated a large herbivore attack by flicking the tree 10 times 1m below the focal internode with a custom-built flicking machine (Appendix B, Figure B.1) that produced 10 flicks at a constant rate and force over 30 seconds. Vibrational disturbance was scored as the number of times workers crossed the focal internode during the first minute after the first flick, subtracted by the baseline (the average number of times workers crossed per minute during the three minutes immediately preceding the first flick).

Response to intruder – *Azteca* ants often protect their trees from the threat of herbivory by *Atta* leafcutter ants and other herbivores (Vasconcelos and Casimiro 1997). In contrast to responding to large-scale vibrations, deterring a leafcutter scout is an easier, less risky task. After only a few bites from responding workers, the leafcutter ant often

jumps off the tree and is unlikely to recruit her nestmates. If the leafcutter scout is permitted to recruit, the *Cecropia* tree could suffer major defoliation (Vasconcelos and Casimiro 1997). Additionally, leafcutter ants likely do not offer much nutritional value and are not captured for consumption. Response to leafcutter ants likely indicates colony aggressiveness as it pertains to host plant defense.

To assess the colony's response to scouting leafcutters, I gently introduced a single *Atta colombica* worker to the focal internode. I scored response to intruder as the number of times workers crossed the focal internode during the first minute after the leafcutter made contact with the stem, subtracted by the baseline. All *A. colombica* workers were of similar size and collected from a foraging trail of a single colony.

Response to leaf damage – *Azteca* ants are known to recruit to fresh damage to the leaves of their host plant (Agrawal and Dubin-Thaler 1999). Free-living species of *Azteca* are among the most carnivorous arboreal ants (Davidson 2003), and thus incidentally benefit plants by consuming insect herbivores. *Cecropia* leaf damage is caused by a variety of insects (Schupp 1986) and *Azteca* workers responding to the damage are occasionally able to immobilize, dismember, and consume palatable herbivores as an additional source of protein (Dejean et al. 2009, Mayer et al. 2014). Many parasitoid wasps use volatile chemicals released by leaf damage as cues to find their caterpillar hosts (Paré and Tumlinson 1999). It is possible that chemicals released by the leaf damage serve as a cue for potential prey, and thus a colony's response may indicate motivation to capture prey.

To assess the colonies' response to leaf damage, I used a standard hole-puncher to make 6 holes in the distal tip of the largest lobe of one of the host plants' leaves.

Response to leaf damage was scored as the maximum number of workers on the damaged leaf within 10 minutes after the damage was discovered. Colonies that did not discover the leaf damage in one or both trials ($n = 3$) were excluded from the consistency analysis.

Exploratory tendency – Colonies may gain new potential territories when branches from surrounding *Cecropia* plants come in contact with their host tree (PRM, pers. obs.). To measure colony exploratory behavior, I provided a new territory by gently placing a dowel rod 1 cm x 85 cm in length in contact with the host tree at the focal internode. I scored exploratory tendency as the total number of times workers ventured past the first 2 cm of the dowel rod in 10 minutes.

Colony demography and leaf damage

After completing the behavioral trials, I harvested the host trees and extracted entire colonies in August of 2013. To subdue and collect the ants, I used internal and external insecticides. The ants chew through most of the internode septa (Longino 1991a), providing a path for the insecticide to traverse the internal height of the tree. I drilled a hole into the base of each tree and inserted the nozzle of a propane-powered insecticide fogger (active ingredient: resmethrin) and discharged the insecticide for several minutes. The tree was then cut at the base, laid on a plastic tarp, and sprayed with a liquid insecticide externally (active ingredients: pyrethrins, piperonyl butoxide, and permethrin). Stems were cut in meter-long segments and split vertically to access the internal workers, brood, and queen. I collected all workers from the stems, leaves, tarps,

and bags and immediately placed them in 95% ethanol. To survey colony size, workers were spread out on grid paper, photographed, and counted using ImageJ software.

Azteca ants colonize *Cecropia* trees as saplings (Perlman 1992), so plant and colony age are likely tightly correlated; hence, I used estimated plant age as a proxy for colony age. *Cecropia* internodes have a consistent growth-periodicity internode branching pattern that allows for accurate estimates of plant age (Zalamea et al. 2012). I counted the number of internodes between branching points of larger, mature trees to estimate an average annual internode output for each *Cecropia* species. I divided the number of internodes from the focal plants by the annual output to estimate plant age.

To assess leaf damage, all leaves were separated and photographed against a light background. Damaged leaf edges were reconstructed using Adobe Photoshop CS6 software. I analyzed leaf damage using ImageJ software (adapted from O'Neal et al. 2002) by calculating the total defoliated area: leaf area without damage (holes filled in via software) subtracted by the leaf area with damage (holes not filled in). When assessing leaf damage, I did not include holes punched for the leaf damage behavioral assay, because I aimed to measure only damage due to natural causes. I presume the majority of missing leaf area is due to defoliating herbivores.

To assess canopy closure, I took photos with a circular fish-eye lens aimed vertically 1.3 m above each plant stump after plants were cleared. I converted images to black and white and used MATLAB to calculate the percentage of black pixels (methods in Korhonen and Heikkinen 2009).

Statistical analyses

Colony consistency for each behavioral trait was analyzed by regressing the scores from the first and second trial and calculating repeatability using the intraclass correlation coefficient (ICC) (Lessells and Boag 1987). The ICC gives the proportion of total variation due to differences among subjects and therefore the degree of subject consistency. For patrolling behavior, where I attained four replicates per colony, I analyzed repeatability using a repeated measures ANOVA. Relationships among traits were analyzed by entering the average scores for significantly consistent traits into an unrotated principal component analysis (PCA). For the leaf damage assay, three colonies required special handling. Two discovered the leaf damage on only one trial, hence I used each colony's single observation rather than the average of two observations. One colony discovered the damage on neither trial. To avoid missing data in the PCA, I assigned this colony the mean leaf damage response value of all the other colonies (as in Manson and Perry 2013). I also performed a PCA without this colony to assess its role in the overall model. To select which components to retain, I used the Kaiser-Guttman stopping rule, which drops components with eigenvalues less than the mean eigenvalue (Jackson 1993). As I describe in the results, this left us with only the first principal component (PC1), which I defined as the colony 'behavioral type.' To examine possible drivers of behavioral difference, I tested for linear correlations of behavioral type with colony age and size. To assess the relationship between ant behavior and host plant health, I tested for linear correlation between leaf damage and each consistent behavioral trait, as well as overall colony behavioral type. Given the distinct contexts of the behavioral traits

(detailed above), I treated these correlations as distinct *a priori* hypotheses and therefore did not correct for false discovery rates (Ruxton and Beauchamp 2008). I also used the behavioral type scores to split the colonies into two categories; “aggressive” (those with positive scores) and “docile” (those with negative scores). I compared the defoliated area between these groups with a Mann-Whitney *U* test. I tested for effects of *Cecropia* species on colony behavioral type using an ANOVA. All statistical analyses were completed using Stata 12.1.

Results

Behavioral consistency

Colonies differed substantially in their response to all assays (range of colony averages for patrolling: 36 – 493 crossings; disturbance: 0 – 633 crossings (Appendix B, Video B.1); intruder: 0 – 123 crossings; leaf damage: 12 – 133 ants; exploration: 0 – 39 crossings). Colony responses also differed consistently in all behavioral traits except exploratory tendency (patrolling activity: Figure 2.1, $n = 14$, $p < 0.001$, ICC = 0.52; vibrational disturbance: Figure 2.2a, $n = 14$, $p < 0.0001$, $r^2 = 0.75$, ICC = 0.86; response to intruder: Figure 2.2b, $n = 14$, $p < 0.05$, $r^2 = 0.34$, ICC = 0.48; response to leaf damage: Figure 2.2c, $n = 11$, $p < 0.01$, $r^2 = 0.59$, ICC = 0.72; exploratory tendency: Figure 2.2d, $n = 14$, $p = 0.71$, $r^2 = 0.01$, ICC = -0.19).

Behavioral correlations

A principal component analysis including the four consistent traits showed that the first component (PC1) explains 48.3% of the variation with an eigenvalue of 1.93 and that all the traits' loading scores are strongly positive (Figure 2.3, Appendix B, Table B.1). Omitting the colony with missing leaf damage response data did not alter the structure of the model (Appendix B, Table B.2). The second component's eigenvalue was barely greater than the mean (1.06) and was therefore excluded from further analysis. I used colony score on PC1 to characterize each colony's behavioral type along a docile-aggressive axis, with higher values indicating greater activity, aggression, and responsiveness. Colony behavioral type was not correlated with colony size (Figure 2.4a, correlation, $n = 14$, $p = 0.18$, range: 1,880 – 13,534 workers) or estimated colony age (Figure 2.4b, correlation, $n = 14$, $p = 0.80$, range: 1 – 4.5 years). Host plant defoliation showed no significant correlation with patrolling ($n = 14$, $p = 0.26$), vibrational disturbance ($n = 14$, $p = 0.06$), or leaf damage response ($n = 14$, $p = 0.31$), but it was negatively correlated with response to intruder ($n = 14$, $p < 0.05$, $r^2 = 0.30$). Defoliation was also negatively correlated with overall colony behavioral type ($n = 14$, $p < 0.05$, $r^2 = 0.33$). I split colonies into docile (negative values, $n = 8$) and aggressive (positive values, $n = 6$) categories (Figure 2.5). The total area of host plant defoliation was significantly lower for aggressive colonies (Figure 2.5, Mann-Whitney U , $n = 14$, $p < 0.05$). Colony behavioral type was not correlated with *Cecropia* species (ANOVA, $n = 14$, $p = 0.46$) or canopy closure (correlation, $n = 14$, $p = 0.35$).

Discussion

Our results support the existence of a collective behavioral syndrome for colonies of *Azteca constructor*. I found that colonies differ substantially and repeatably in four distinct behavioral traits, all of which were positively related. Thus, each colony could be characterized by a behavioral type score along a docile-aggressive axis, with higher scoring colonies being more active, responsive, and aggressive than those with lower scores. Furthermore, colony behavioral types were correlated with their host plants' health such that trees containing more aggressive colonies also exhibited less leaf damage.

Exploratory behavior was not consistent, which suggests colonies display flexibility under certain conditions. Furthermore, colonies tend to have a higher exploratory score in the first trial (Appendix B, Figure B.2), which may indicate colony desensitization, habituation, or another form of learning. Under certain conditions, a single *Azteca* colony can occupy multiple *Cecropia* trees that have adjacent stems or overlapping leaves and reap the benefits of both plants (PRM, pers. obs.). New potential territories, such as overlapping *Cecropia* leaves, are often already occupied by other *Azteca* colonies and encounters among non-nestmates result in a fight to the death (PRM, pers. obs.; Adams 1990). Colonies with high rates of exploration risk losing workers to gain potential resources, but this trade-off remains to be investigated. The fact that exploration behavior was not consistent over time highlights the importance of assessing a range of colony traits because some behaviors have more plasticity than others.

What causes this behavioral syndrome? Our data discount two obvious explanatory factors: colony age and size. As colonies mature, changes in the resources they need might be reflected in their collective behavior (Bengston and Jandt 2014), but I found no correlation between estimated colony age and behavioral type. This may reflect the purposefully narrow age range of our colonies, and it remains possible that age affects behavioral differences over larger age differences. Nonetheless, age does not appear to explain the behavioral variation that I observed. Another potential explanation for this variation is that more active colonies have more workers (as in seed-harvester ants; Waters et al. 2010). However, I show that total colony size is independent of colony behavioral type, suggesting that colonies invest differently in the number of workers afforded to a given stimulus. Other studies documenting collective behavioral syndromes in social insect colonies either control for colony size (Wray et al. 2011, Blight et al. 2016) or find colony size independent of behavioral type as well (Bengston and Dornhaus 2014), suggesting that there is a general need for an alternative explanation for behavioral variation. Environmental effects, such as local climatic conditions, colony density, or resource availability, likely play a role in selecting for one behavioral type over another, or for variation itself (for examples, see Pinter-Wollman et al. 2012; Pruitt and Goodnight 2014; Bengston and Dornhaus 2014; Bengston and Dornhaus 2015). Personality in *Azteca* colonies may also be influenced by resource availability provided by their host plant, such as internal nesting space and food body production, a topic which merits further research.

Differences in colony personality can also be interpreted as distinct life history strategies along the r-/K-selection continuum. In this framework, more bold, aggressive,

and active individuals are associated with r-type strategies; faster resource accumulation, faster growth, earlier reproduction, and shorter life spans (Biro and Stamps 2008). In *Temnothorax* ants, colonies with risk-taking personality types are associated with r-selected life-history strategies in that they grow faster and invest more in reproduction than colonies with risk-averse personalities (Rother et al. 2016). Risk-taking colonies are found at higher latitudes with shorter growing seasons, which likely drives the selection for a fast-paced, r-type life-history strategy (Bengston and Dornhaus 2014). In the context of the *Azteca-Cecropia* mutualism, r-type aggressive colonies may deter more threats and provide better protection for their host tree, but the maintenance of high vigilance and loss of workers may be metabolically costly (Riechert 1988). The energetic demand could be met by higher rates of prey-capture and food body production from a healthier host-plant, which could promote faster growth for both partners and reinforce the strategy. On the other hand, K-type docile colonies may conserve energy by hiding within the walls of their host plant, allowing defoliating herbivores to damage their tree, which could decrease growth rates in both partners and delay reproduction. While colony life-history strategy may reflect inherent colony differences (e.g., genetics and development), it may also depend on host-plant investment strategies (e.g., tradeoffs between food body production and vertical growth), or environmental variation (e.g., light level and soil nutrients).

It is well established that there is a significant difference in *Cecropia* leaf damage between plants with and without *Azteca* ants (Schupp 1986), but here I document a significant difference among *Azteca*-occupied plants. Colonies with positive behavioral type scores are associated with extremely and uniformly low levels of leaf damage,

appearing to be very effective at controlling their host plants' exposure to herbivory. In contrast, host plants housing colonies with negative behavioral type scores have a high variation in leaf damage, suggesting the plants are subjected to greater risk. Furthermore, by comparing correlation coefficients, colony behavioral type explains leaf damage variation better than any single colony trait that I tested – including response to leaf damage. This suggests that it is important that colonies have a strong response in several distinct contexts to limit herbivory. Response to intruder also significantly correlated with host plant defoliation, highlighting that a strong, immediate response to encountering an intruder may outweigh the response to an already damaged leaf. Defoliation can be detrimental to plant growth, competitive ability, and fitness, especially in the tropics (Coley and Barone 1996). For example, individuals of the tropical plant *Piper arieianum* that have more leaf damage suffer from a long-term decrease in growth, seed production, and seed viability (Marquis 1984). I did not measure how fitness is shaped by defoliation rates, and future studies are needed to assess how the behavioral type of the inhabiting colony influences the success of its host plant, which has implications for partner selection and fidelity (Mayer et al. 2014).

While I document the relationship between colony personality and plant health, it is important to underscore that the directionality remains uncertain - it is possible that the amount of host plant leaf damage influences colony behavioral type. In other ant-plant systems, plants can alter the output of their extrafloral nectaries in response to herbivory, but it is still poorly understood how food body production might change (Mayer et al. 2014). External factors such as intraspecific genetic variation (Marquis 1984) or variation in local herbivore abundance (Coley and Barone 1996) could give rise to differential leaf

damage rates. Reduction in photosynthetic area from defoliation may decrease food body production since Müllerian food bodies are largely carbon-based (Rickson 1971). Colonies with access to fewer food bodies may have lower levels of activity and aggressiveness. This possibility gives a novel insight into mechanisms that maintain behavioral syndromes in natural populations. Colony behavior and plant health may influence each other in a feedback loop; aggressive colonies help prevent leaf damage and are rewarded with more food bodies, making them even better equipped to defend their host plant, whereas less aggressive colonies permit more defoliation and suffer lower resource availability. Behavioral differentiation in host plant defense has been documented between different plant-inhabiting species (Mayer et al. 2014), but not within species. Our results imply that partner-host dynamics, cost-benefit analyses, and conflict in ant-plant mutualisms may be more complicated than previously thought. The correlation between colony personality and leaf damage must ultimately be backed by controlled experiments, and further research is required to elucidate factors contributing to the important ecological relationship between colony behavior and host plant health.

Our study on collective behavioral syndromes of a social insect is the most comprehensive investigation conducted entirely in the field, and the first of its kind pertaining to mutualisms. I show that colony personality is a robust, ecologically relevant phenomenon that cannot be explained by colony size or age, and that it is an important consideration for mutualism dynamics. The current study paves the way for future research on the internal and external factors contributing to the variation among colonies and its relationship within the mutualism.

Figures

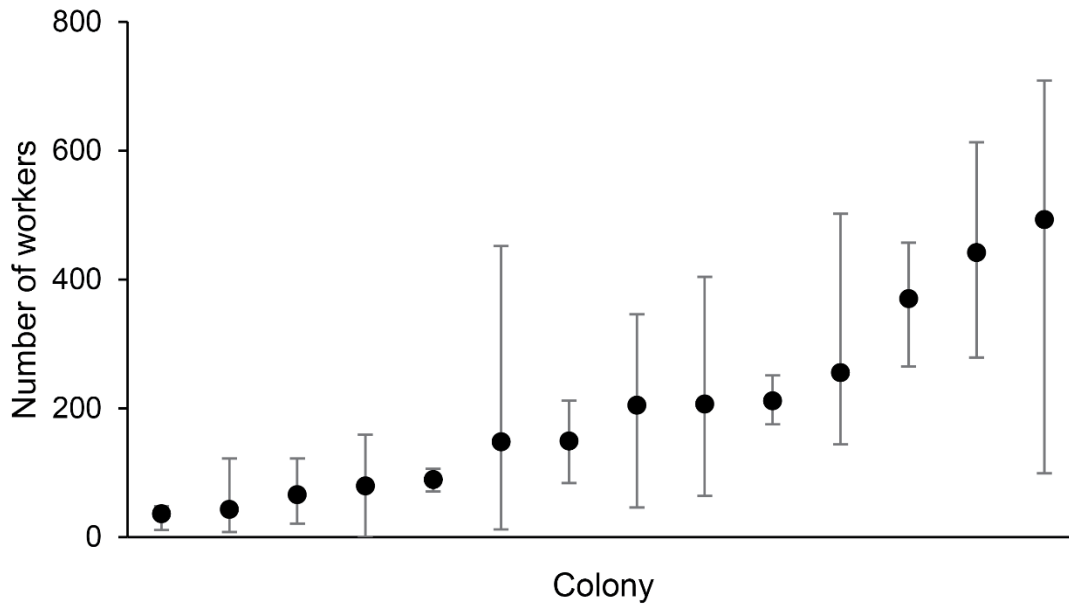


Figure 2.1. Colonies differed repeatably in their patrolling behavior, shown as the number of workers crossing the lowest leaf internode in five minutes. Points indicate the colony mean and error bars indicate the range.

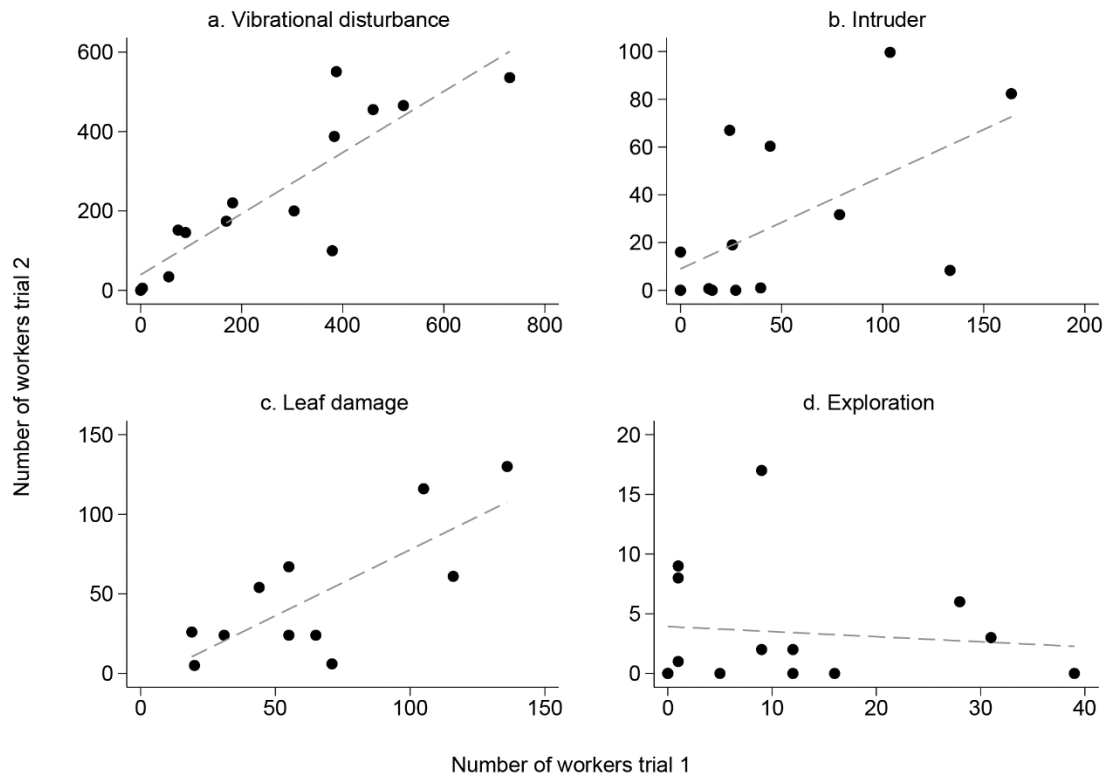


Figure 2.2. An overview of colony consistency for four traits shows significant regressions of the first trial on the second trial. Colonies differed repeatably in **a.** vibrational disturbance, **b.** intruder response, and **c.** leaf damage response, but not in **d.** exploratory tendency.

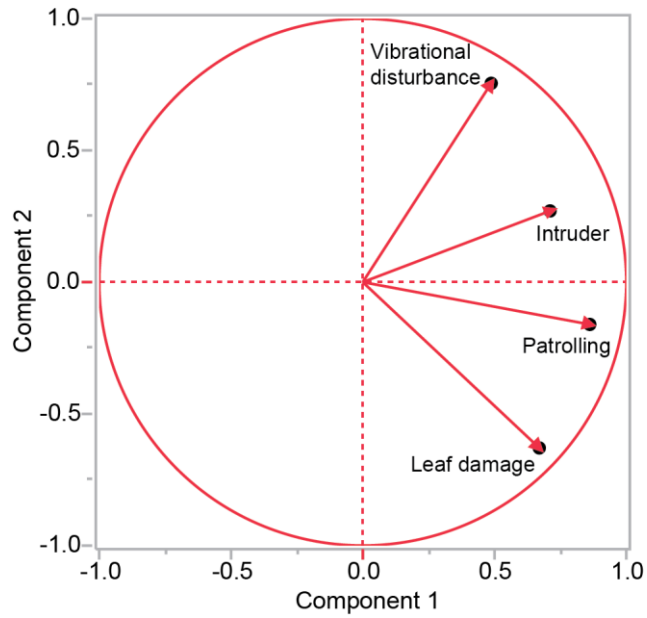


Figure 2.3. A principal component analysis of the four consistent traits shows that the first component explains 48.3% of the variation and that all the traits load strongly positive. Colonies' first principal component scores were used as the colony "behavioral type" scores.

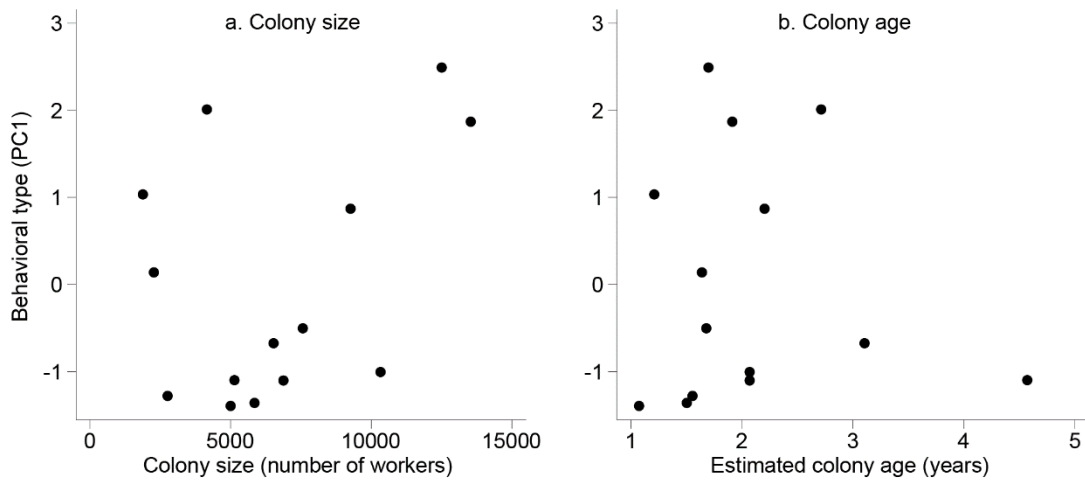


Figure 2.4. Behavioral type was not correlated with colony size (a) or age (b). Colony size was measured by harvesting the host plant, extracting the workers, photographing them, and using imageJ software to mark and count each one. Colony age estimates were determined by estimating host plant age.

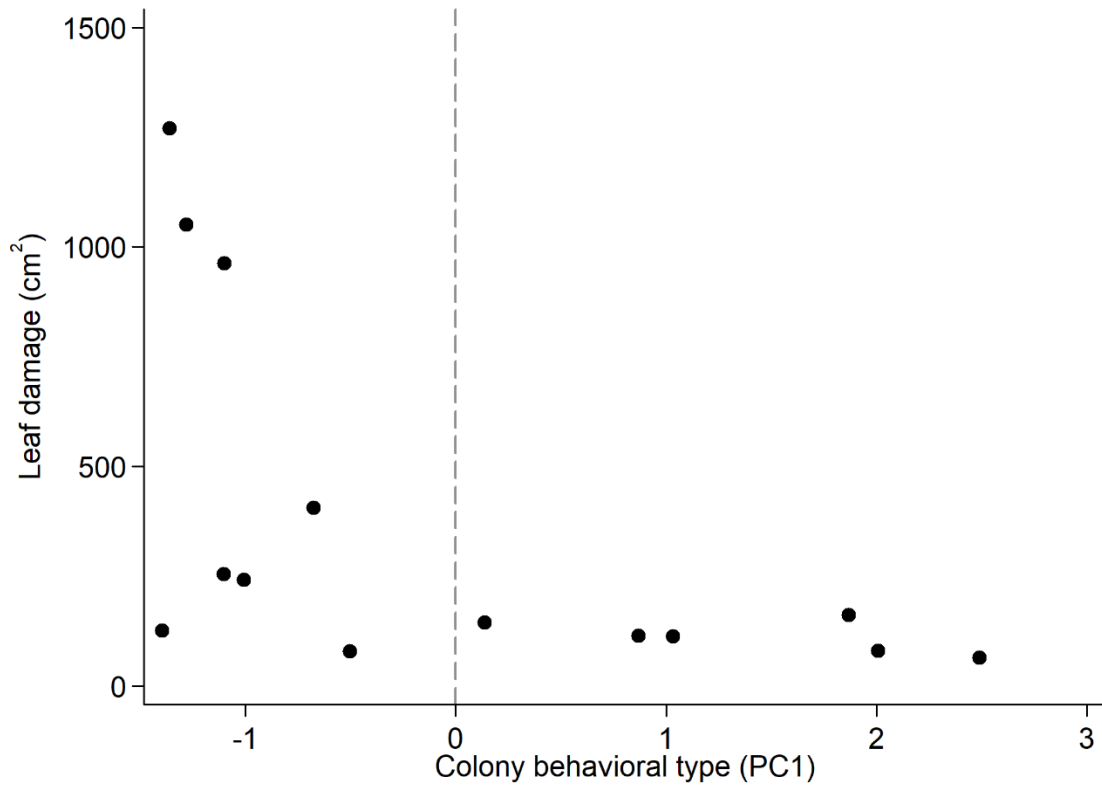


Figure 2.5. The total amount of host plant leaf damage differed between colony behavioral types. The dashed line represents where colonies were split into aggressive and docile categories.

CHAPTER 3
ANT-PLANT SOCIOMETRY

Abstract

For ant colonies that obligately nest within plant hosts, aspects of their sociometry are likely intertwined with their host plant, which has implications for the strength and stability of the mutualism. In the *Azteca-Cecropia* mutualism, tree structure determines where the colony nests in the stem's hollow, segmented internodes. Little is known about how the colony is distributed and organized and how this might affect the colony's ability to respond to threats or opportunities. These ants also express collective personalities that may be influenced by host tree dimensions, colony distribution within the tree, worker body size. We investigated patterns of and relationships among five major categories of sociometry; plant growth, colony growth, colony structure, and collective behavior. In the following paragraphs, we outline driving questions for each sociometric category through the lens of the mutualism. There was a high level of among-colony variation in vertical nest distribution within the host plant. Colony distribution correlates more strongly with colony size than tree structure, and ant body size correlates more strongly with tree structure than colony size. Colony personality was independent of colony distribution and tree structure but correlated with ant body size such that less polymorphic colonies had more aggressive personalities. The results of this study provide insights into how ant-plant structural relationships may contribute to plant protection and the strength of the mutualism.

Introduction

To understand how social insect colonies function as superorganisms, it is essential to quantify patterns of colony growth, nest architecture, and life cycle, a field of study known as insect sociometry (Tschinkel 1991). The relationships and scaling between colony traits give insight about development, collective physiology, evolutionary constraints, and plasticity. Such basic natural history is often overlooked or lacks detail and depth because data can be hard to collect.

For ant colonies that obligately nest within plant hosts, aspects of their sociometry are likely intertwined with their host plant, which has interesting implications for the strength and stability of the mutualism. We studied ant-plant sociometry in *Azteca constructor* colonies nesting in *Cecropia* trees in the lowland tropics of central Panama. *Cecropia* trees provide hollow internodes for nesting and glycogen-rich food bodies for the ants, which in return protect the trees from herbivores and vines (Janzen 1969, Rickson 1971, Schupp 1986) and provide nitrogen enrichment (Sagers et al. 2000, Dejean et al. 2012, Oliveira et al. 2015). This system provides a unique and interesting view of insect sociometry because the complex environmental factors that typically shape sociometry – habitat structure, resource abundance, territory size, interactions with intruders, microclimate – are simplified through the colony's interaction with their host plant. The host plant *is* their environment; a biotic environment shaped by coevolution with the ants themselves. We investigated patterns of and relationships among five major categories of sociometry; plant growth, colony growth, colony structure, ant morphology,

and collective personality. In the following paragraphs, we outline driving questions for each sociometric category through the lens of the mutualism.

What is the relationship between colony growth and plant growth? Comparing colony growth rate to that of the host plant reveals potential strains in the mutualism. If plant growth outpaces colony growth, ants may not be able to keep up with herbivory pressure (Pringle et al. 2012), and plants suffer from leaf damage and a reduction in fitness. If colony growth outpaces plant growth, the benefit from ant protection diminishes as domatia-growing costs or food nourishment increase (Fonseca 1993, 1999). If growth rates are equal, there is likely positive feedback between colony and plant growth, and mutualism is reinforced (Frederickson and Gordon 2009).

How do colony structure and organization relate to host plant form? The physical nest architecture of plant-ants is determined by their host plant's hollow nesting spaces, called domatia. Colonies make decisions about which domatia to occupy, how to distribute themselves within the plant, and whether to add structural elements. How a colony is distributed and organized may influence the colonies' ability to forage, tend brood, respond to threats, and communicate effectively. Little is known about how plant-ant colonies distribute and organize themselves within their host plant, and what forces influence these patterns. The dissection of a large, mature *Cecropia* tree revealed that the majority of the *A. constructor* colony is centralized in a large bulge in the main trunk (Longino 1991a), suggesting that the colony's distribution may remain static as the tree grows. By expanding the sample size and including smaller trees, we can address a series of interesting questions. How are colony components – specifically workers, queen, brood, scale insects, refuse piles, carton, and entrances – distributed in the tree, how are

these components spatially related to one another, and how does the distribution change with tree growth?

Do host plants influence worker morphology? Worker size and polymorphism are often associated with sociometric measures, such as colony size, age, and annual cycle (Tschinkel 1988, 1993, Murdock and Tschinkel 2015, Kwapich et al. 2017). The degree of polymorphism within a colony depends on intrinsic factors (genotype and development), external factors (environment and enemies) or a combination of both (nutrition and social environment) (Wills et al. 2018). In ant-plant mutualisms, worker morphology might be related to mutualism dynamics or physical traits of the host plants themselves, especially since colony performance feeds back into plant fitness. In the Sonoran desert, ant species with larger body size are associated with more myrmecophyte species (Chamberlain and Holland 2009), suggesting that they can take advantage of a wider range of resources. A comparison of two plant-ants found that larger body size and greater variation in body size were associated with host plants having larger domatia and prostomata (Meunier et al. 1999), suggesting that worker morphology may coevolve with plant traits. In addition to plant morphology, worker size may match the size of the dominant herbivores threatening their host. Ant species that invest in worker number over worker size may be better at scrutinizing the surface of their host plant and removing small sap sucking insects (Gaume et al. 1997), but worse at fending off larger insects and vertebrates. Worker morphology has been quantified in the non-*Cecropia*-inhabiting congener *Azteca trigona* (Adams 1994), but has not been formally described in *A. constructor* until the present study.

How is collective personality related to nest organization, worker morphology, and tree structure? Colonies of *A. constructor* display collective personalities along a docile-aggressive axis for a suite of behavioral traits (Marting et al. 2018). The sources of behavioral variation are not clear, but are likely to lie at the intersection of environment and genetics (Sih et al. 2015, Bengston et al. 2018). Aside from colony size, few studies have examined the relationship between colony personality and sociometric traits. Semi-permanent traits like nest architecture likely effect colony behavior over long periods of time. The physical attributes of nest entrance chambers influence collective behavior by affecting worker encounter rates (Pinter-Wollman 2015) or ability to exit the nest in a state of alarm (Burd et al. 2010). Colony personality may also interact with worker morphology. If more aggressive colonies have smaller workers, there may be an energetic tradeoff between producing larger workers or increasing colony vigor. Alternatively, if worker size increases with colony aggression, it may reflect an energetic alignment. Furthermore, larger workers may enable colonies to be more aggressive because workers can cover more ground and respond faster. In the context of an ant-plant mutualism, colony personality and plant traits may be related. Plants provide two major resources for their ant colonies – nesting space and food bodies, both of which are correlated with plant height (Folgarait and Davidson 1995). Higher resource availability may increase colony energy reserves, fueling higher activity and aggression. However, the causality may flow in the opposite direction. In the *Azteca-Cecropia* mutualism, colonies with more aggressive personalities live in trees with less leaf damage (Marting et al. 2018), which may increase plant growth. Finally, colony aggression and plant growth may influence each other in a positive feedback loop, stabilizing the relationship.

To address these questions, we harvested 14 trees containing colonies with known personality scores (Marting et al. 2018) and measured the number of workers, queen, brood, scale insects, refuse piles, carton, and entrances in each internode to determine how colonies were vertically distributed. We then measured the morphology of a subset of workers from each colony. In addition, we measured key features of host tree morphology, including tree height, diameter, number of internodes, number of leaves, and leaf area. We first use these data to describe the patterns of each separate sociometric category (plant size, colony size, colony organization and structure, and collective personality), then we explore the relationships among them, focusing on the degree to which colony sociometry is intertwined with host plant biology.

Methods

Focal species and study site

Cecropia trees are diecious pioneer plants with a single central stem that produces a new leaf-bearing, hollow internode every 2-4 weeks (Berg et al. 2005). The giant, radial leaves produce Müllerian food bodies at specialized sites called trichilia at the petiole-stem juncture for about 3 months before dying and detaching (Davidson 2005). After 3-5 years, branches grow out from the central stem and bifurcate annually to produce a candelabra structure (Alvarez-Buylla and Martinez-Ramos 1992, Sposito and Santos 2001, Zalamea et al. 2008). Workers chew entrances to individual internodes and holes between the internodes' septa, creating a nearly complete, internal passageway throughout

the length of the tree (Longino 1991a). Workers can further partition the available volume by constructing carton galleries inside the internodes (Longino 1991b), made from a combination of regurgitated plant materials including parenchyma, a soft, white tissue lining the inside of newly formed internodes (Valverde and Hanson 2011). In a related species, *Azteca brevis*, carton material is structurally reinforced by a multi-species network of fungal hyphae (Mayer and Voglmayr 2009). Dark brown “refuse piles” can be found throughout the internal structure, and may be important for cultivating nematodes (Esquivel et al. 2012) or fungus (Nepel et al. 2016, Mayer et al. 2018), or for nutrient transfer. Colonies display distinct behavioral tendencies, or personalities, in that they differ repeatably in a suite of behavioral traits that are independent of colony size and age (Marting et al. 2018).

We located 14 *A. constructor* colonies along a 12 km stretch of Pipeline Road in and around the lowland tropical rainforests of Soberania National Park, Colón, Panama between March and May 2013. At this site, there are four common *Cecropia* species (*C. peltata*, *C. obtusifolia*, *C. longipes*, and *C. insignis* and three common *Cecropia*-inhabiting *Azteca* species (*A. constructor*, *A. alfari*, and *A. isthmica*). All pairings of ant and tree species can be found, but *C. peltata*, *C. longipes*, and *A. alfari* tend to be found in large disturbed areas, while the others tend to be found in forest gaps (PM, personal observation). For the purposes of this study, we focused on a single *Azteca* species (*A. constructor*) that occupied *C. obtusifolia* (n = 10), *C. peltata* (n = 2), and *C. insignis* (n = 2).

Colony founding in *Azteca* involves secondary monogyny, meaning multiple queens cooperate in the incipient stages, and eventually fight to the death until one queen

remains (Perlman 1992). To avoid these complex intracolony dynamics, we attempted to select trees old enough to have a single queen (above 2m tall). Trees can reach over 20m in height and have many branching points, but we used shorter trees (below 8m tall) with single stems for assay standardization and ease of access. Therefore, our sampling reflects the sociometry of juvenile trees.

Tree measurements

We measured tree height, diameter, and number of leaves upon harvesting the colony. To assess total leaf area, all leaves were separated and photographed against a light background. To assess canopy closure, we took photos with a circular fish-eye lens aimed vertically 1.3m above each plant stump after plants were cleared. We converted images to black and white and used MATLAB to calculate the percentage of black pixels (methods in Korhonen and Heikkinen 2009).

Cecropia internodes have a consistent growth-periodicity internode branching pattern that allows for accurate estimates of plant age (Zalamea et al. 2012). We counted the number of internodes between branching points of larger, mature trees to estimate an average annual internode output for each *Cecropia* species. We divided the number of internodes from our focal plants by the annual output to estimate plant age. *Azteca* ants colonize *Cecropia* trees as saplings (Perlman 1992), so plant and colony age are likely tightly correlated.

Colony size, structure, and vertical distribution

After completing the behavioral trials, we harvested the host trees and extracted entire colonies in August of 2013. To subdue the ants and minimize disturbance to their internal distribution, we used internal and external insecticides. The ants chew through most of the internode septa (Longino 1991a), providing a path for the insecticide to traverse the internal height of the tree. We drilled a hole into the base of each tree and inserted the nozzle of a propane-powered insecticide fogger (active ingredient: resmethrin) and discharged the insecticide for several minutes. The tree was then cut at the base, laid on a large plastic tarp, and sprayed with a liquid insecticide externally (active ingredients: pyrethrins, piperonyl butoxide, and permethrin). Stems were cut in meter-long segments and split vertically to access the internal colony. For each internode, we quantified the internal domatium dimensions, the number of workers, brood (larvae and pupae not distinguished), scale insects, and refuse piles, and noted the presence of the queen, entrances to the exterior, carton material, and leaf-baring petioles. After we quantified the internal distribution of the colony, we collected all workers from the stems, leaves, tarps, and bags and immediately placed them in 95% ethanol. To survey colony size, workers were spread out on grid paper, photographed, and counted using ImageJ software.

Ant morphology

For each colony, we selected a subset of 100 workers from a large vial of ethanol containing the entire colony. To reduce size bias selection as much as possible, we mixed the ethanol in to a vortex with forceps and selected workers haphazardly. For each ant, we separated head, mesosoma, gaster, and legs, and arranged them on an index card using double-sided tape. With a camera mounted on a dissection scope, we photographed each ant using SPOT software. We calibrated the images with a micrometer scale that was included in each photograph, and measured head width and mesosoma length using ImageJ software.

Behavioral traits

To characterize colony-level behavior, colonies were subjected to five bioassays: patrolling behavior, vibrational disturbance, response to intruder, response to leaf damage, and exploratory tendency (detailed in Marting et al. 2018). Colonies received each assay at least two times to assess behavioral consistency (patrolling behavior assay was repeated four times per colony). To standardize behavioral measurements across different tree sizes, we focused on the central stem at the lowest leaf's internode, which we estimated to be the location of median colony distribution based on four preliminary tree dissections. For patrolling behavior, vibrational disturbance, and response to intruder, we scored activity by counting the number of times we saw a worker completely traverse the lower septum line on the external surface of the focal internode, regardless of

direction or identity. For leaf damage assays, we focused on an entire leaf instead of the stem and counted the number of workers on that leaf every minute. Trials were recorded with an HD camcorder (Panasonic HC-X900M) between May and August of 2013. The results of these behavioral analyses are published in Marting et al. 2018, but we use this data to test new relationships in this study.

Statistical analyses

Data were analyzed with correlation, linear regression, ANOVA, and paired t-test. We log-transformed certain variables to evaluate allometric scaling by testing if the observed scaling coefficient (log-log slope) differed from the predicted scaling coefficient. The predicted scaling coefficient for isometry was calculated as the exponent of the dependent variable (e.g., length = 1; area = 2; volume = 3) divided by the exponent of the independent variable. Observed scaling coefficients that were the same as predicted indicated isometric relationships, below indicated negatively allometry, and above indicated positively allometry. In the figure notes, “Iso-slope” indicates the predicted scaling coefficient of an isometric relationship, “Slope” indicates the observed scaling coefficient, and “Slope-p” indicates the p -value resulting from a Wald test comparing the predicted and observed scaling coefficients.

We used principal component analysis to simplify the characterization of each of the five major categories of sociometrical data (tree size, colony size, colony structure, worker morphology, and colony personality). We performed separate unrotated PCA for each category, to reduce its multiple defining traits to a single variable. These summary

variables were then used to investigate relationships among the categories. All statistical analyses were performed in Stata 12.1.

Results

Plant size and growth patterns

The *Cecropia* trees we sampled ranged from 2.42-7.95m tall with 55-144 hollow internodes that provided a total internal volume of 0.23-5.65L with an estimated age range of 1-4.5 years. Only the oldest tree bore inflorescences during the study (*C. insignis*, 2 inflorescences). Leaf area and tree height scaled with negative allometry such that every 10-fold increase in height produced a 7-fold increase in leaf area (Figure 3.1). Total leaf area was driven more by an increase in leaf size rather than leaf number (Figure 3.2). Tree height and estimated age were not correlated (regression, $n = 14$, $p = 0.47$).

Colony size and growth patterns

All associated colonies of *A. constructor* were monogynous and ranged in size from 1,880-13,534 workers, with 73-93% of the workforce on the external surface of their tree at the time of harvesting. Alate production was low, with only 2 of the larger colonies producing 1-22 males and no females. The number of brood and number of

workers scaled with negative allometry such that with every 10-fold increase in workers, there was only a 4-fold increase in brood (Figure 3.3).

The total number of workers scaled isometrically with tree height (Figure 3.4a) but was not correlated with estimated tree age (correlation, $n=14$, $p = 0.918$). Furthermore, the number of external workers increased isometrically with total leaf area (Figure 3.4b).

Nest structure and vertical distribution

We detail nest structure and vertical distribution for an exemplar colony in Figure 3.5. Colonies occupied 27-62% of the available internodes. The vertical distribution of workers depended on the *Cecropia* species they inhabit (ANOVA for proportional height of median workers, $p < 0.05$, Figure 3.6), with *C. peltata* supporting a low, broad distribution, *C. insignis* supporting a high, narrow distribution, and *C. obtusifolia* ranging between the other two. To compare vertical distribution patterns across different tree and colony sizes, we rendered the proportion of each nest component by tree height decile (Figure 3.7). Internal tree volume was not evenly distributed vertically, but steadily increased with decile height. Nearly all leaves were in the top half of the tree, with leaf proportion steadily increasing with decile height therein. The proportion of workers, brood, scale insects, and refuse piles peaked around the 8th and 9th height decile. Carton was more evenly distributed, tapering off in the lowest deciles, while the proportion of entrances steadily increased with decile height.

Tree height predicts median leaf height, median leaf height predicts median worker height, median worker height predicts median brood and queen height (Figure 3.8a). The relative median height (percent of tree height) of these components is independent of tree height, i.e., the various tree components are at the same proportional location in the tree, regardless of the tree's absolute height (Figure 3.8b). Median leaf height was above median worker distribution height, while median workers height was generally above brood, carton, and refuse median height. There was no difference between median worker height and scale insect height or queen height (paired t-test, Figure 3.9).

Carton was found in $32\pm 4\%$ (mean \pm s.e.) of total plant internodes and $53\pm 6\%$ of ant-occupied internodes (Figure 3.10). Internodes with carton contained $66\pm 5\%$ of internal workers and $82\pm 4\%$ of the brood (Figure 3.10).

We ran a principal component analysis to determine the relationship among different nest components in individual ant-occupied internodes, which revealed 2 significant factor axes that together explain 50% of the variation: a leaf-entrance-hemipteran-refuse axis, and a brood-queen-carton axis (Table 3. 1, Figure 3.11).

Compared to an average ant-occupied internode, the royal chamber (internode containing queen) was more likely to contain carton and brood, and less likely to contain an entrance or refuse piles (Figure 3.12).

Worker size and allometry

Workers were varied in size with head widths ranging from 0.57-1.29mm and were positively allometric (log-log slope = 1.13, Figure 3.13).

Relationships among sociometric categories using PCA

For each of the five major categories of sociometrical data – tree size, colony size, colony structure, worker morphology, and colony personality, we selected the defining traits (detailed below) and performed separate unrotated principal component analyses (Table 3.1). For every PCA, the first principal component was the only eigenvalue greater than the mean (Jackson 1993) and explained most of the variation. Furthermore, the nature of the loadings on PC1 were easily interpreted and given intuitive summary descriptors.

For tree size: height, total internal volume, total leaf area, and stem diameter all loaded strongly positive and PC1 explained 90% (Table 3.2). We named PC1 “tree size” because higher values indicate taller trees with greater diameter, internal volume, and leaf area. For colony size: total workers, brood, hemipterans, and refuse piles all loaded strongly positive and PC1 explained 71% (Table 3.3). We named PC1 “colony size” because higher values indicate colonies with more workers, brood, hemipterans, and refuse piles. For colony distribution structure: queen, worker, and brood height loaded strongly positive, while worker and brood breadth loaded strongly negative and PC1 explained 74% (Table 3.4). We named PC1 “colony distribution breadth” because higher

values indicate that the colony nest components have narrower distribution and are located higher in the tree. For worker morphology: allometry slope, head size disparity, max head width, and average head width all loaded strongly positive and PC1 explained 80% (Table 3.5). We named PC1 “worker size” because colonies with higher values have larger workers, greater size disparities, and steeper allometries. The results for colony personality were published in Marting et al. 2018 (see Chapter 2), but we list them here for congruency. Vibrational disturbance, leaf damage, intruder, and patrolling all loaded strongly positive and PC1 explained 48% (Appendix D, Table 3.D.1). We named PC1 “colony personality” and colonies with higher values were more active, aggressive, and responsive. Five numbers described each colony: the PC1 score for each sociometrical category (Figure 3.14).

We used colony PC1 scores to test for correlations among all sociometrical categories. No correlations were significant except those shown in Figure 3.15. Larger trees supported larger colonies ($p = 0.02$, Figure 3.15a). Larger colonies promoted broader nest distributions ($p = 0.008$, Figure 3.15b). Worker morphology was not correlated with colony size, but instead correlated with tree size such that larger trees supported larger, more allometric worker morphologies ($p = 0.02$, Figure 3.15c). Colonies with larger, more allometric worker morphologies tended to be less aggressive ($p = 0.06$, Figure 3.15d).

Discussion

Our results support the notion that the growth and distribution of *Azteca constructor* colonies are intertwined with their *Cecropia* host plants. Costs to the host plant can accrue if tree growth outpaces colony growth (Pringle et al. 2012) or vice versa (Fonseca 1993), but our results show that, over the size range that we sampled, colony and plant growth rates appear to be similar, which reinforces the stability of the mutualism. Furthermore, colony size increased isometrically with tree height, but not with tree age, which suggests that there is positive feedback between colony and plant growth rates. Older trees were not necessarily taller, which likely reflects that some plants are growing in unfavorable conditions, e.g., poor soil nutrients or low light, which in turn affects colony growth. Additionally, the number of workers on the external surfaces, i.e., the stem, leaves, and petioles, increased isometrically with total host plant leaf area, suggesting that ant density remains consistent as the tree grows. Leaf damage did not increase with tree size, but rather decreased with colony-level aggression (Marting et al 2018), suggesting that colony behavior is more important for preventing herbivory than colony size. As leaf size increases, the most effective patrolling strategy may shift. Further research is merited to test whether colonies employ different collective search strategies as their host plant surfaces increase.

Vertical worker distribution tended to be most dense near the top of the tree, which reflects the distribution of available nesting space food body-bearing leaves. The median leaf height was consistently above the median worker height, and median brood height was below the median workers height. This suggests that as leaves grow, workers

follow and brood lags behind. Even though less than half of internodes contained carton galleries, we found that the majority of workers and most of the brood reside in internodes with carton, suggesting they serve as brood storage. The shape of *Azteca* distributions which resembled the distribution patterns of several ground-nesting ant species (Murdock and Tschinkel 2015), which may be due to comparable resource proximity. However, this similarity may only be superficial.

Given the distribution shape and height of each nest component, we posit a generalized hypothesis about how the colony distributes itself as the tree grows: as trees grow upward, adding new leaves and larger internodes, workers quickly chew entrances and move into the new space, harvest the new food bodies, and bring the scale insects to feed on the softer tissues. Carton is built more slowly and eventually brood is deposited there. Lower internodes are eventually abandoned, leaving behind used carton and sealed up entrances (workers must actively maintain the entrance sites by chewing, or the tree will eventually seal them). This hypothesis is limited to the range of tree sizes included in this study. It appears that colony distribution patterns may shift dramatically as tree's central stem bifurcates into several branching points. In the dissection of a larger tree in Costa Rica, the *A. constructor* colony distribution appear to be very centralized, with the queen and all brood residing in a large, permanent, carton-filled bulge near the center of the tree (Longino 1991a). Such a centralized configuration may be advantageous for workers patrolling and foraging across several distributed meristems. Future sampling should include a larger range of tree sizes and structures to capture the transition from a more vertically distributed to a more centralized nest structure.

Despite the generalized pattern, there was a large amount of variation in how colonies distributed themselves within their trees. This variation is partially explained by larger colonies having broader distributions, but other factors not measured here may influence colony distribution. In *Temnothorax* ants, colonies consistently vary in how they structure their nests across time and contexts (DiRienzo and Dornhaus 2017). Our data were snapshots of colony distribution – it would be interesting to test whether patterns of colony distribution are consistent across time or persisted across host plant transplants.

In many polymorphic species, worker morphology correlates with colony size and age, with larger colonies producing larger workers, greater size variation, and steeper allometries (Wills et al. 2018). This trend reflects the natural progression of resource acquisition, colony nutrition, and colony growth. However, here we show that worker morphology is not correlated with colony size, but rather host tree size. Worker size may be controlled by intrinsic factors like nutrition; larger trees may produce more food bodies, more nutrition is invested per larvae, resulting in larger workers. It is also possible that the nutrient ratios of the food bodies shift with tree height, resulting in larger workers. Worker size may also be responding to external factors like available space, load size, or entrance size. Larger trees naturally provide more voluminous chambers, greater surface area, and larger territory to patrol, which could be more efficiently traversed by larger workers. Perhaps larger trees produce larger food bodies that are more efficiently carried by larger workers. Finally, larger trees may have larger prostomata – the dedicated dimpled sites where ants chew entrances into the internal internode space.

Larger-headed workers may fill larger entrance gaps more appropriately to prevent intruders from entering the tree.

Colony personality was independent of colony size, tree size, and vertical distribution. However, an interesting pattern emerged with ant morphology. Colonies with more aggressive personalities tended to have smaller, less allometric worker morphologies. This trend may reflect some resource investment tradeoff – perhaps colonies can either have an aggressive demeanor or larger workers, but not both. Alternatively, worker size may be connected to task demand. Our measures of aggression are based on the number of ants responding to a given stimulus. If the colony has larger workers, perhaps fewer ants need to respond, because they are more efficient at dealing with threats. Another possibility is that colonies fed more food bodies can produce larger workers than colonies not fed enough food bodies. Colonies not fed enough may try to compensate for their nutrient deficiency by increasing prey consumption, thus projecting a more aggressive collective personality. More experiments need to be done.

Our study on ant-plant sociometry is a comprehensive investigation on growth patterns, colony organization and vertical distribution, worker polymorphism, and collective personality. We show that colony sociometry is intimately intertwined with host plant biology, and that is an important consideration for mutualism dynamics.

Figures and Tables

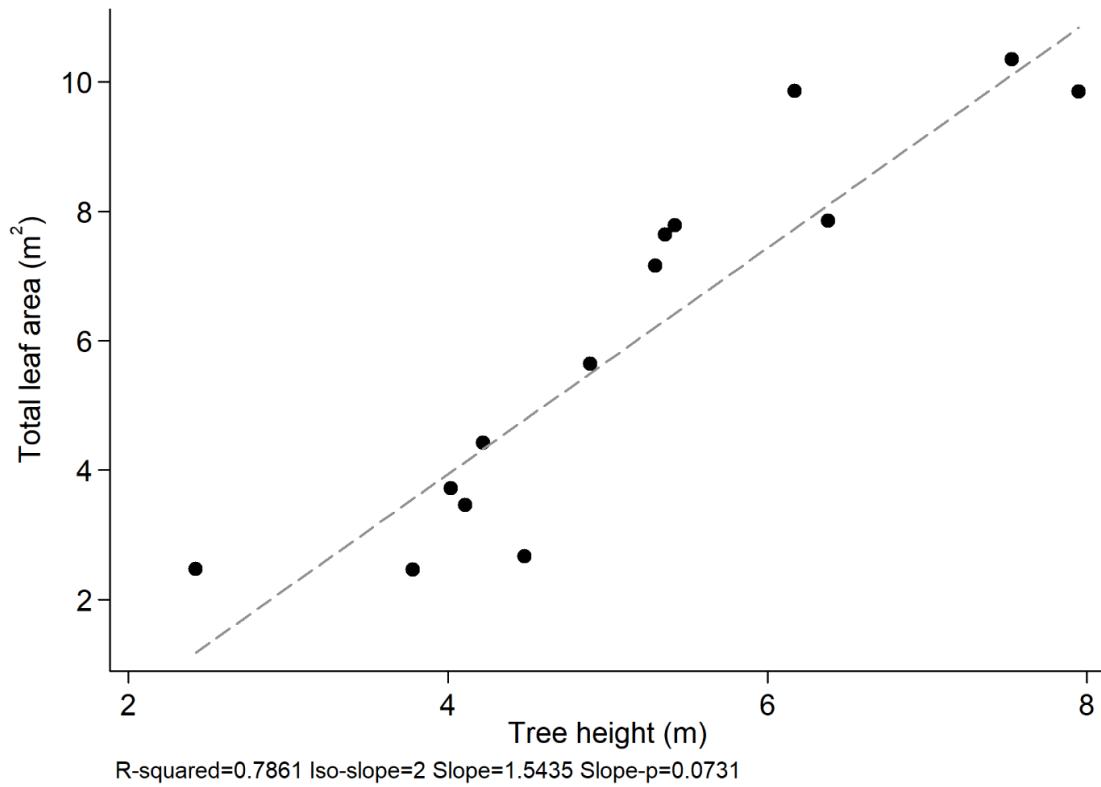


Figure 3.1. The relationship between total leaf area and tree height. The dashed line represents an allometric regression (log-log relationship). The scaling coefficient was nearly significantly lower than the predicted isometric slope of 2 (see *Methods*), indicating an isometric relationship that is almost negative allometric.

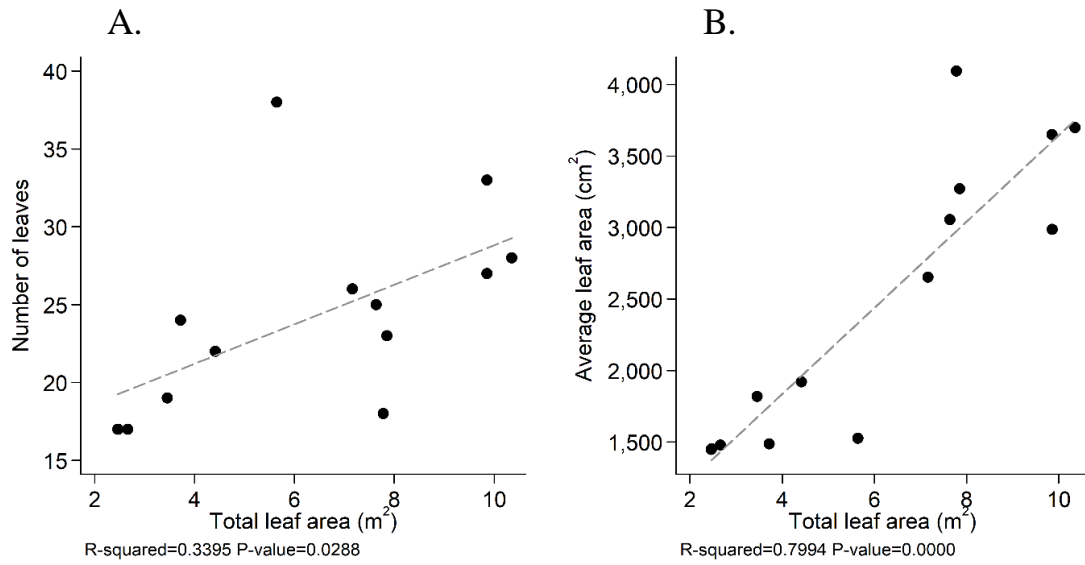


Figure 3.2. Leaf features contributing to total leaf area. A. The relationship between number of leaves and total leaf area. The dashed line represents a linear regression. B. The relationship between average leaf area per leaf and total leaf area. The dashed line represents a linear regression.

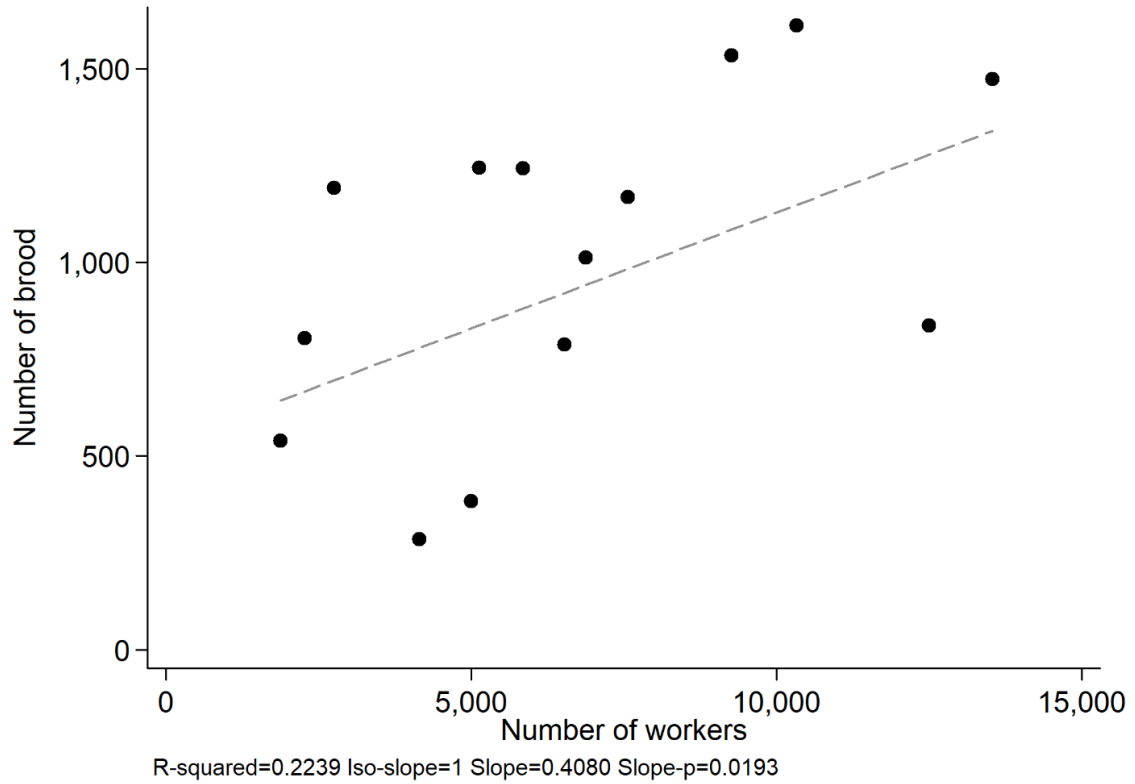


Figure 3.3. The relationship between total number of brood and the total number of workers. The dashed line represents an allometric regression (log-log relationship). The scaling coefficient was significantly lower than the predicted isometric slope of 1, indicating a negative allometric relationship.

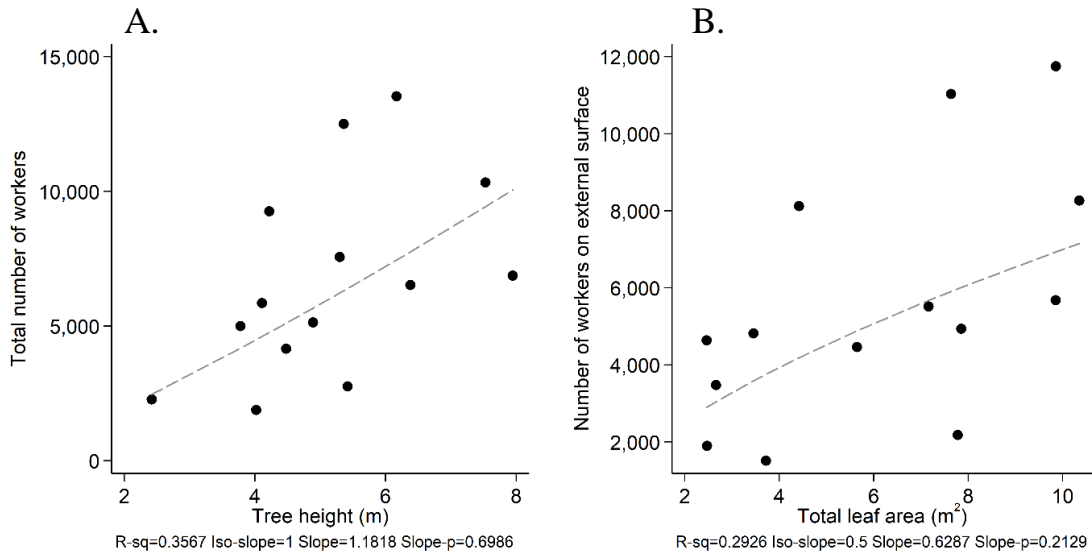


Figure 3.4. How workers scale with tree size. A. The relationship between total number of workers and tree height. The dashed line represents an allometric regression (log-log relationship). The scaling coefficient was not significantly different from the predicted isometric slope of 1, indicating an isometric relationship. B. The relationship between the number of workers on the external surface of the plant and total leaf area. The dashed line represents an allometric regression (log-log relationship). The scaling coefficient was not significantly different from the predicted isometric slope of 0.5, indicating an isometric relationship.

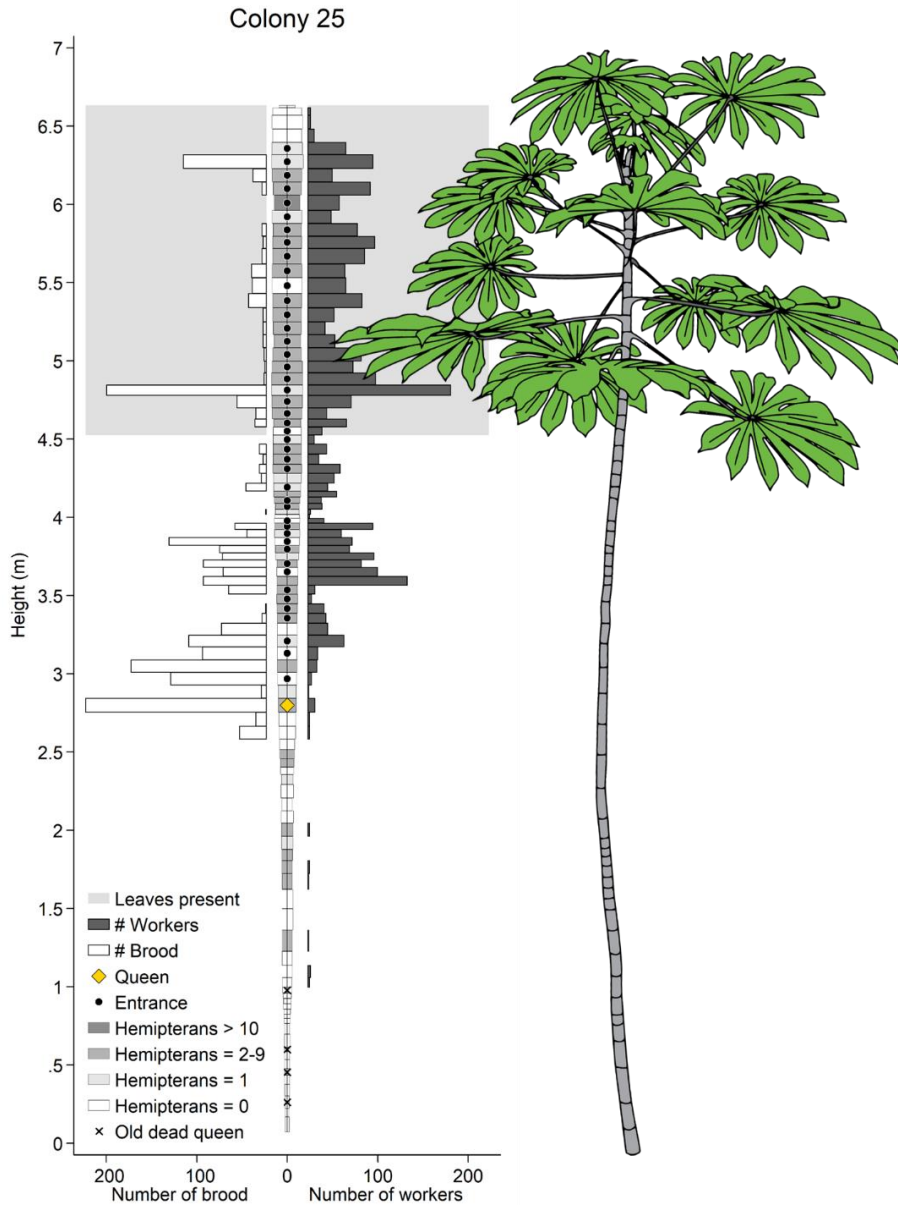


Figure 3.5. The distribution of colony nest components within an exemplar *Cecropia* tree.

Each bar in the central column represents an internode from the central stem, and the dimensions are scaled to the height and width of the internal volume of each internode (width is doubled relative to height to show the components more clearly). The height of the bars to the left represent the number of brood and the bars to the right represent the

number of workers. The shading of each internode indicates the hemipteran density. The shaded area near the top of the tree represents internodes that bore leaves. The location of the queen is indicated by the golden diamond, and entrances are indicated by black circles.

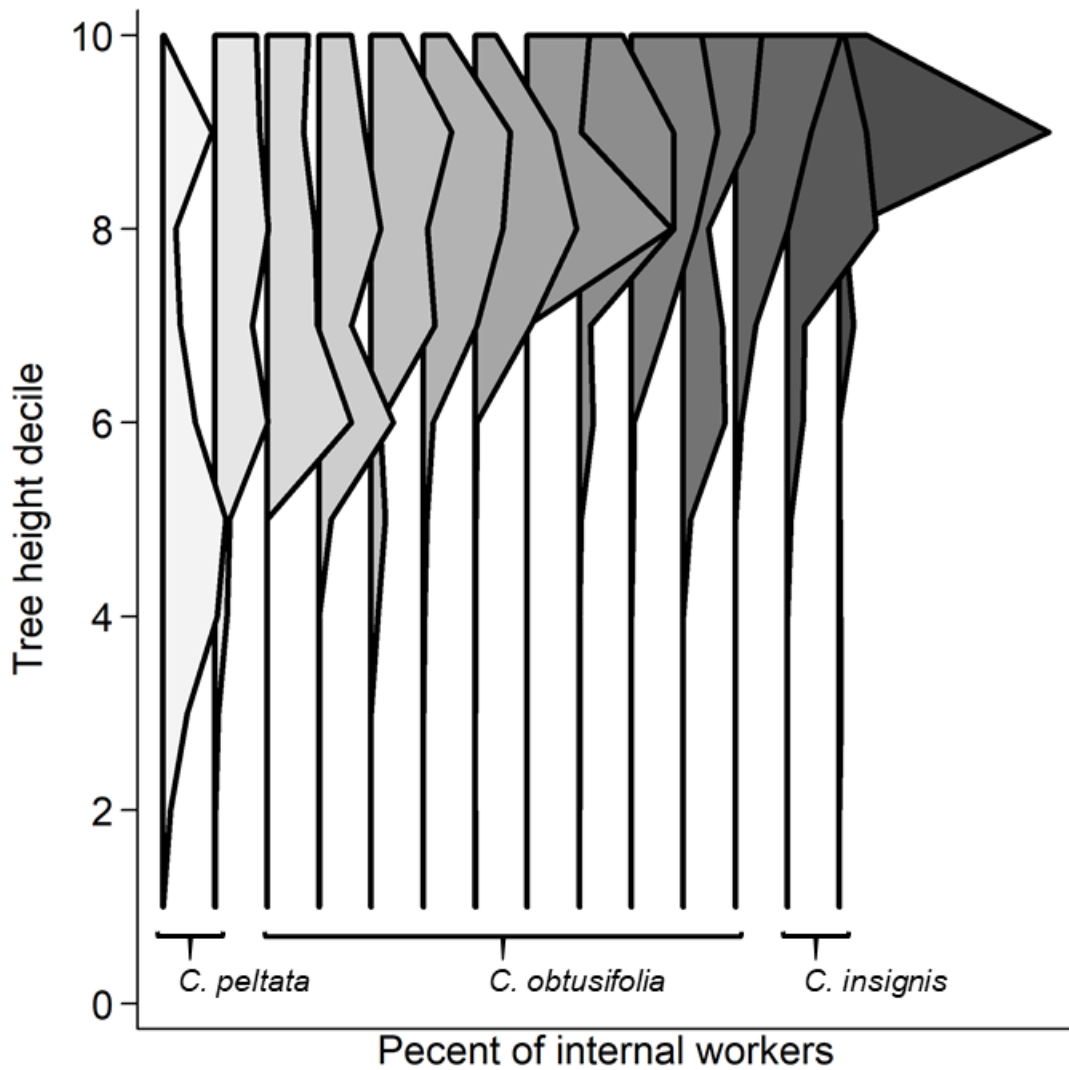


Figure 3.6. The distribution of workers within *Cecropia* trees. The proportion of internal workers are rendered by tree height decile for each tree. Colonies are arranged by *Cecropia* species, then by the proportional height of the median worker distribution. The proportional height of the median worker distribution differed significantly among *Cecropia* species (ANOVA, $F = 7.17$, $p = 0.0101$).

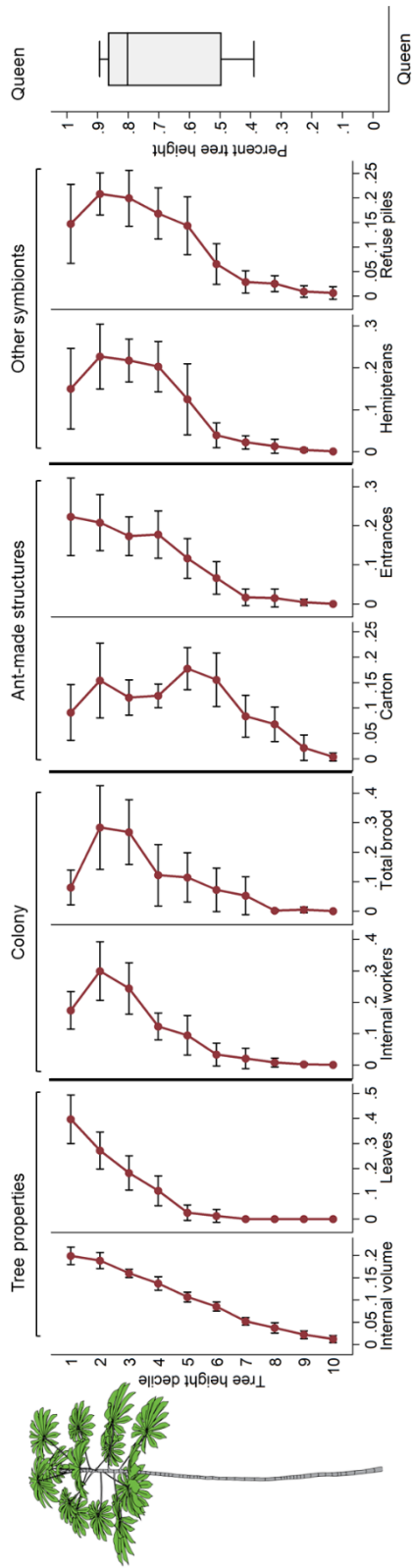


Figure 3.7. The mean proportion of each nest component as a function of tree height decile. Error bars indicate 95% confidence intervals. The box plot represents queen location as a proportion of tree height.

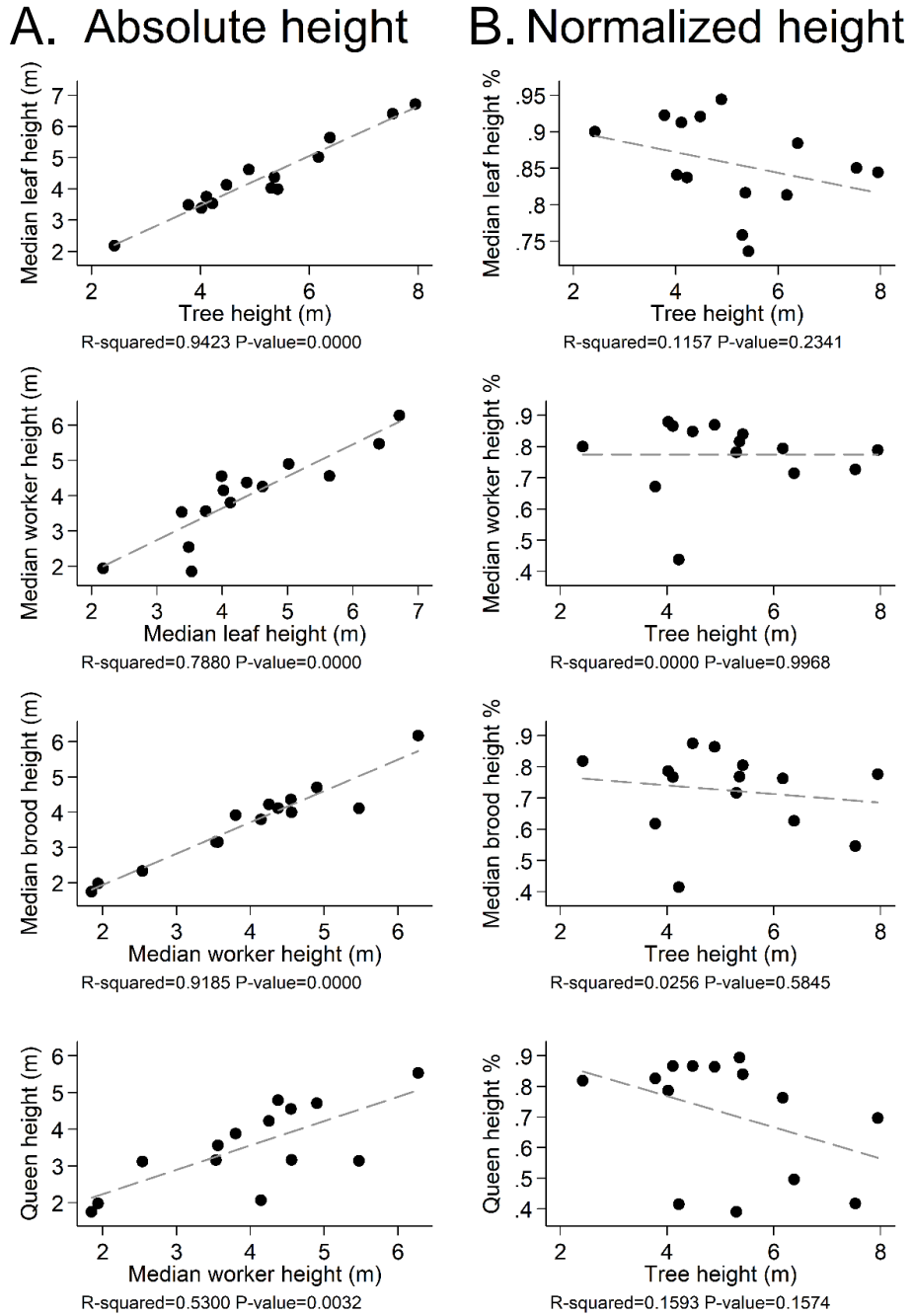


Figure 3.8. The relationship among the median height of tree and colony components. A. The absolute height of each components. B. The proportional height of each component relative to absolute tree height.

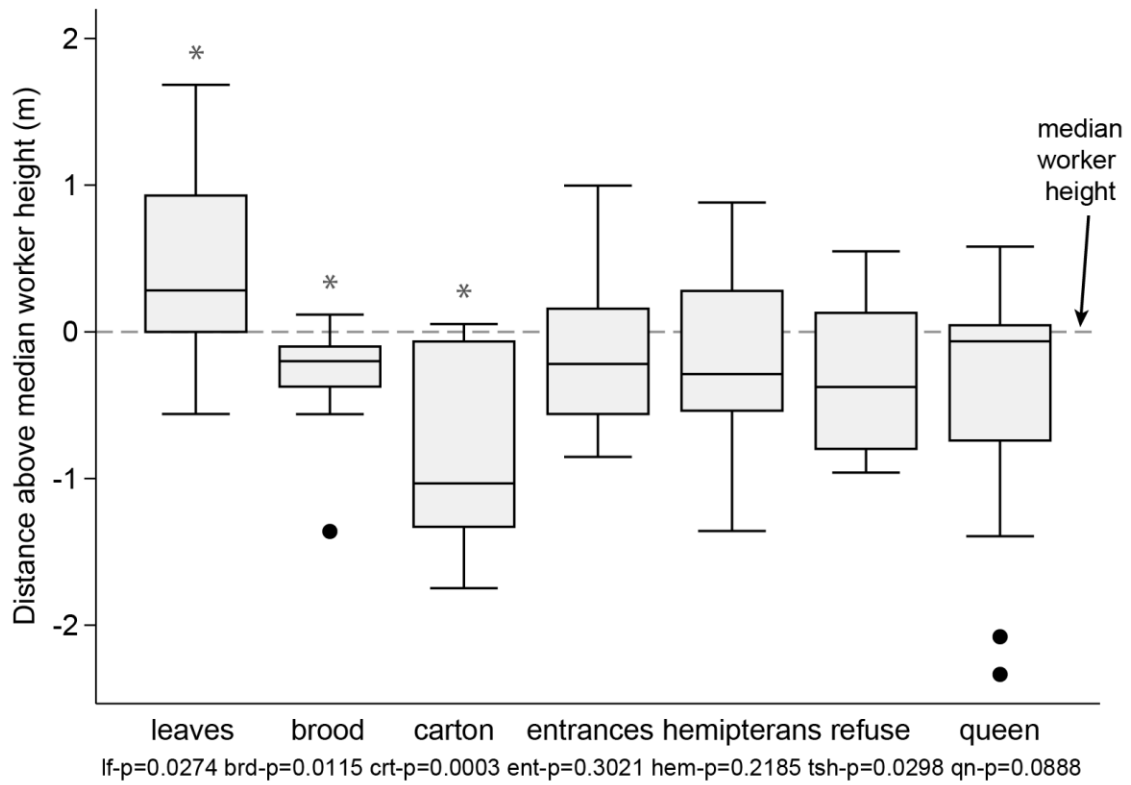


Figure 3.9. The distance between median nest component heights and median worker height. Asterix indicate a significant difference from the median worker height.

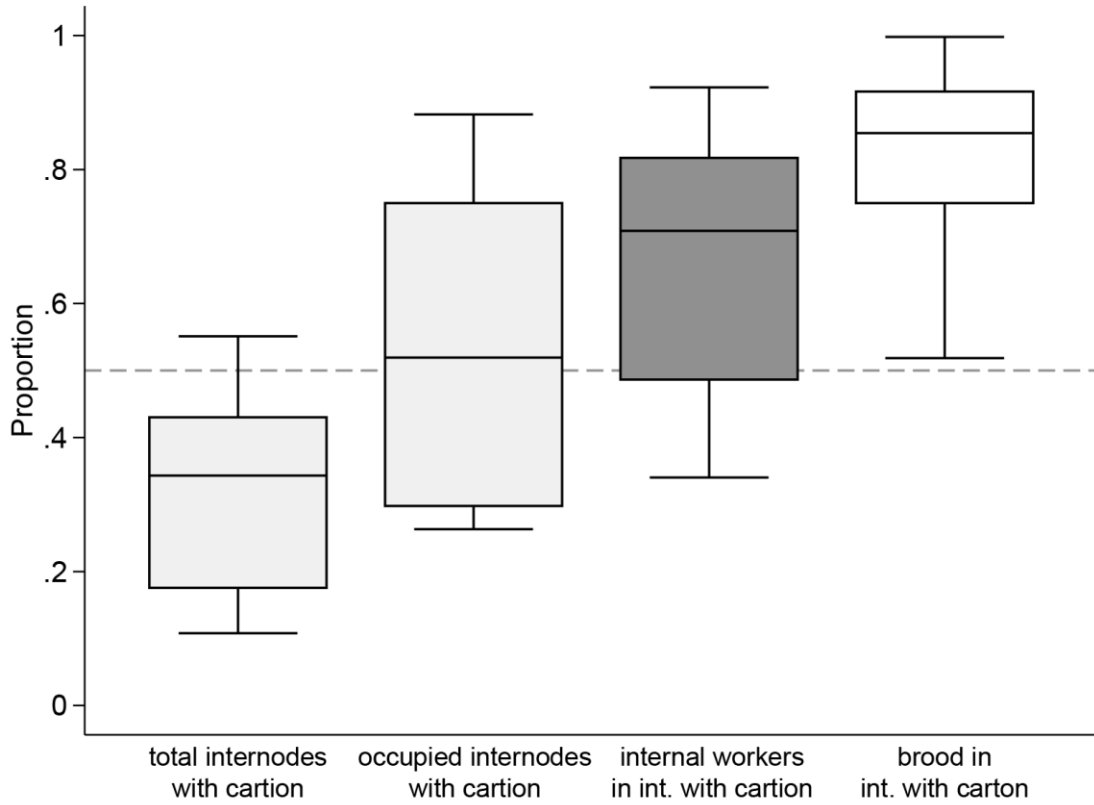


Figure 3.10. A comparison between carton prevalence and carton use. While less than half of the total internodes contain carton, more than half of the total workers and brood reside in internodes with carton.

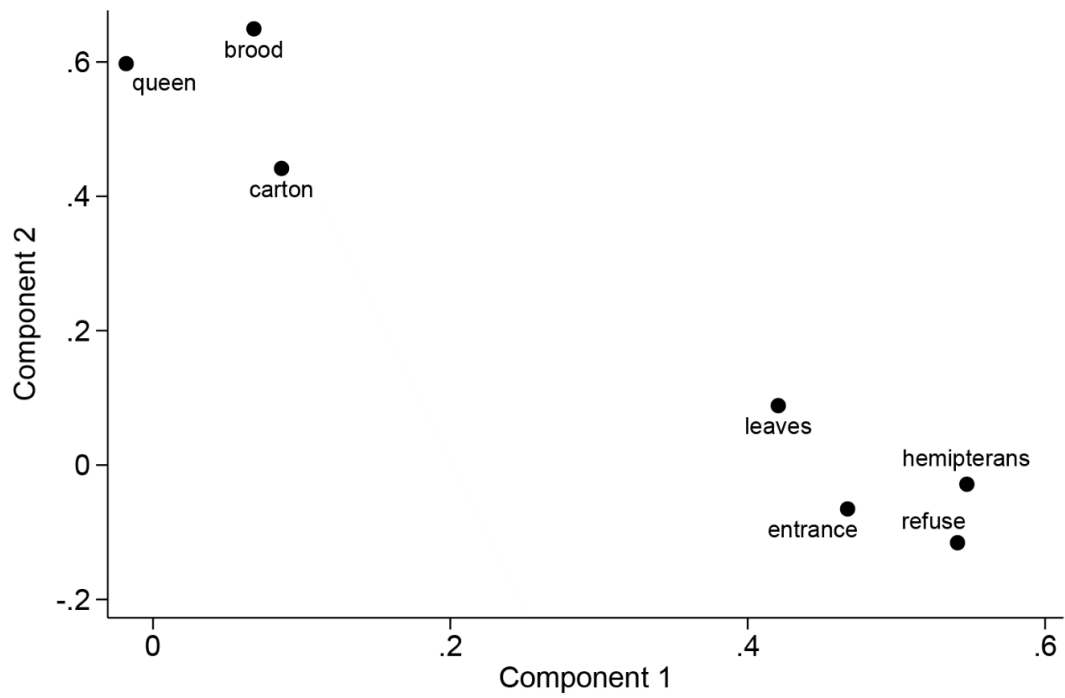


Figure 3.11. The loading scores from a PCA of nest components. The nest components from every occupied internode from every tree were entered in a principal component analysis to determine how they were related.

Table 3.1. A summary of the principal component analyses for the nest components in each internode (n = 613). Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	1.915	1.588
Variance Explained	27.4%	22.7%
Loading Scores		
Number of brood	-	0.6494
Number of refuse piles	0.5411	-
Number of hemipterans	0.5474	-
Entrance present	0.4672	-
Carton present	-	0.4415
Leaf present	0.4206	-
Queen present	-	0.5975

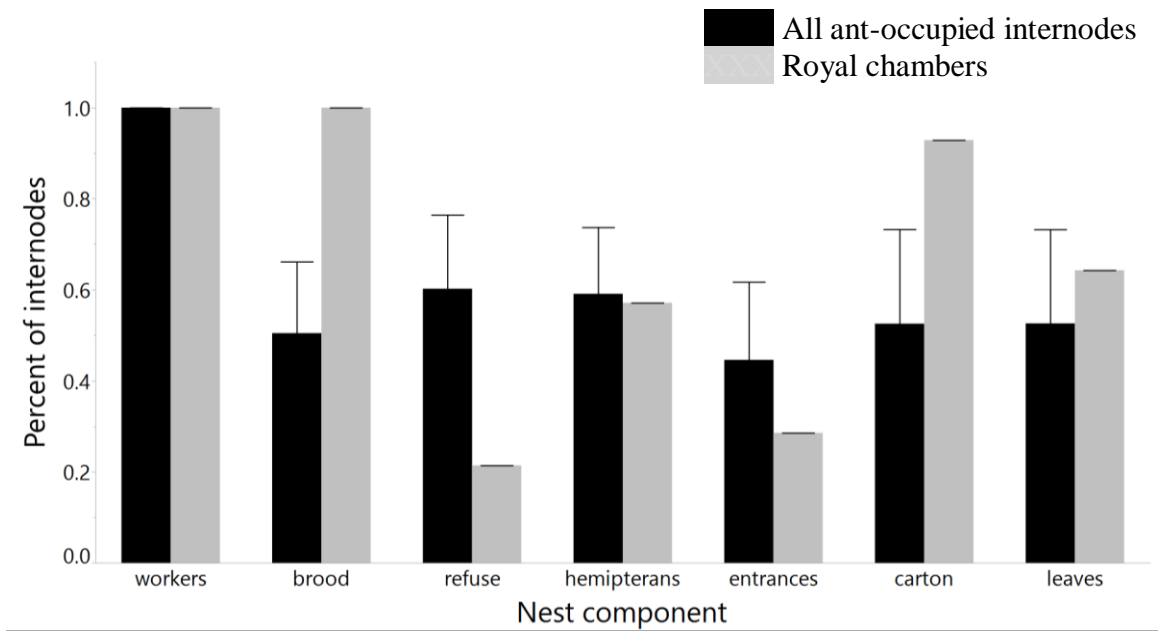
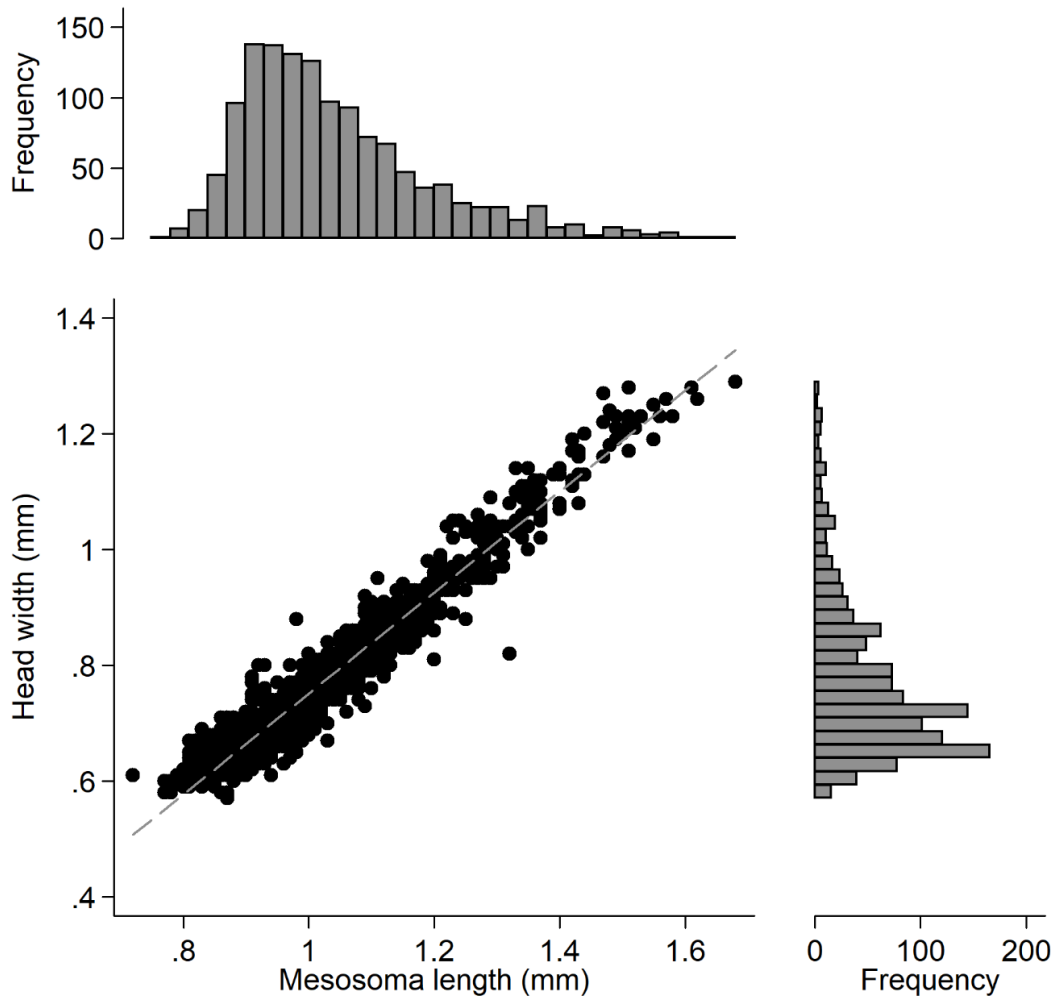


Figure 3.12. Nest components in the royal chamber. Black bars indicate the mean proportion all ant-occupied internodes containing each nest component (presence or absence) per tree (error bars indicate 95% confidence interval). Grey bars indicate the direct proportion of royal chambers that contained each nest component.



R-squared=0.9352 Iso-slope=1 Slope=1.1311 Slope-p=0.0000

Figure 3.13. The relationship between head width and mesosoma length for workers from all colonies ($n = 1,300$). The dashed line represents an allometric regression (log-log relationship). The scaling coefficient was significantly higher than the predicted isometric slope of 1, indicating a positive allometric relationship. The histogram shows the frequency of workers by mesosoma length and head width on their respective axes.

Table 3.2. A summary of the principal component analyses for the tree size. Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	3.674	0.172
Variance Explained	91.8%	4.3%
Loading Scores		
Height	0.5074	-
Diameter	0.5047	0.7443
Internal volume	0.4920	-
Total leaf area	0.4958	-0.6590

Table 3.3. A summary of the principal component analyses for the colony size. Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	2.905	0.532
Variance Explained	72.6%	13.3%
Loading Scores		
Total workers	0.5132	-0.2821
Total brood	0.4449	0.8921
Number of refuse piles	0.5138	-
Number of hemipterans	0.5241	-0.3020

Table 3.4. A summary of the principal component analyses for the colony distribution.

Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	3.710	0.670
Variance Explained	74.2%	13.2%
Loading Scores		
Percent of internodes with workers	-0.4096	0.7000
Percent of internodes with brood	-0.4587	0.3192
Median proportional height of workers	0.4399	0.5715
Median proportional height of workers	0.4985	0.2545
Proportional height of the queen	0.4241	-

Table 3.5. A summary of the principal component analyses for the worker morphology.

Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	3.210	0.451
Variance Explained	80.2%	11.3%
Loading Scores		
Mean head width	0.4866	0.3425
Max head width	0.5241	-0.4595
Max head/min head width	0.5201	-0.4639
Head-mesosoma scaling coefficient (log-log slope)	0.4669	0.6756

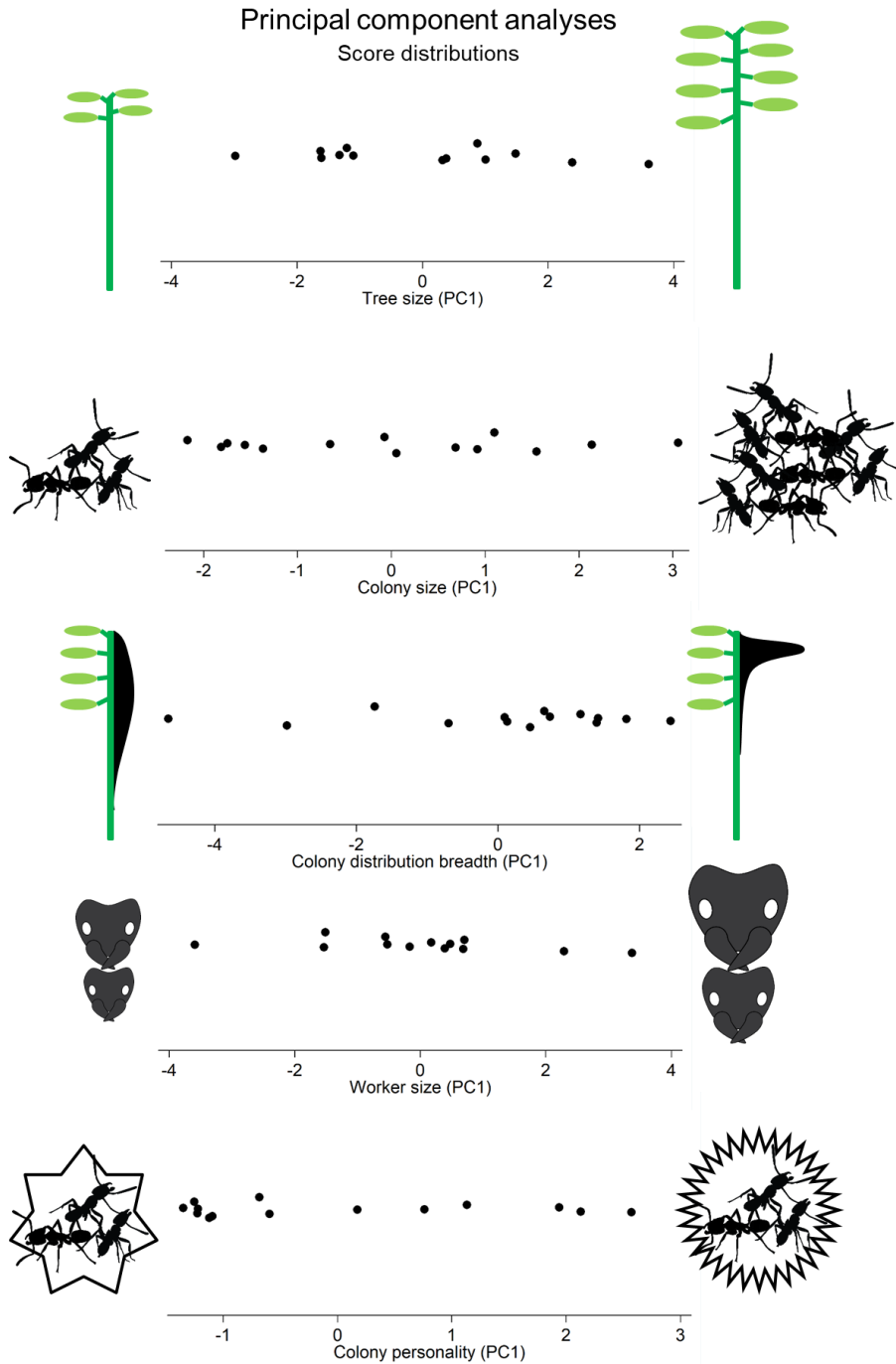


Figure 3.14. Score distributions for the 5 major sociometric categories. Plots display how colonies vary along the PC1 axes for tree size, colony size, colony distribution breadth, worker size, and colony personality. The illustrations on either side are visual interpretations of what the extreme values represent for each PC1.

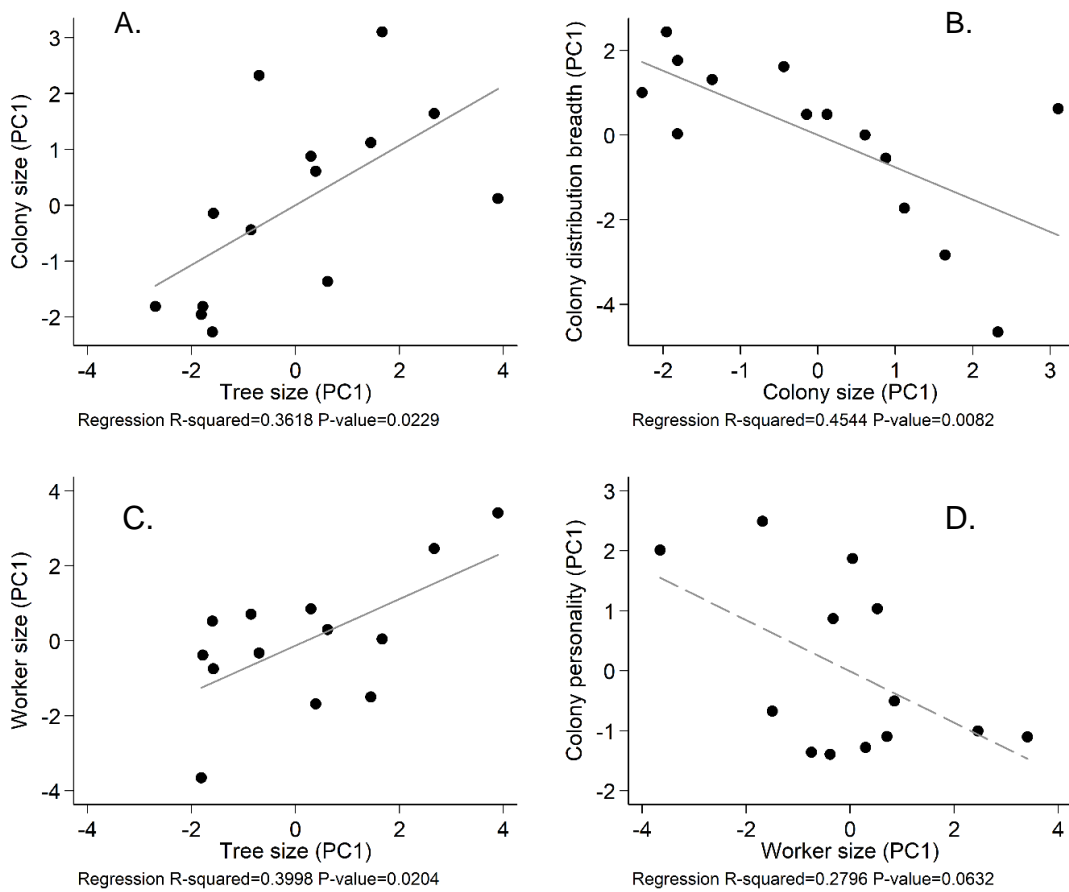


Figure 3.15. Correlations among sociometric categories. Solid lines indicate a significant correlation between traits and dashed lines indicate a nearly significant trend ($p < 0.1$). A. The relationship between colony size and tree size. B. the relationship between colony distribution breadth and colony size. C. The relationship between worker size and tree size. D. The relationship between colony personality and worker size.

CHAPTER 4

COLONY PERSONALITY AND SOIL NUTRIENTS

Abstract

The symbiosis between *Azteca* ants and *Cecropia* trees is one of the most successful and prominent mutualisms of the neotropics. Plants provide food bodies and nesting cavities for ant colonies that protect the plant from herbivores and encroaching vines. However, some colonies are consistently more aggressive than others in a suite of behavioral traits measured in the field, revealing that colonies themselves have personalities. Plants with more active, aggressive colonies have less leaf damage, suggesting that collective personality has ecologically relevant consequences. What is driving the differences in personality types? We designed an experiment to test whether collective personality is a fixed, inherent property of the colony or influenced by environmental factors. At the same time, we tested a more specific hypothesis regarding environmental factors: soil nutrients increase the resources that the plant offers to the ants, which in turn increases colony aggression. We conducted a three-phase experiment where we 1) assessed colony behavior in the field, 2) harvested trees, extracted colonies, and transplanted them into greenhouse plants under differing nutrient treatments, and 3) re-assessed colony behavior 10 months later. Our results strengthen the evidence that colonies of *Azteca constructor* maintain a collective behavioral syndrome along a docile-aggressive axis under natural field conditions, but we show that the structure of this behavioral syndrome unravels when colonies are transplanted to the greenhouse.

Colonies did not behave consistently from the field to the greenhouse, which supports the hypothesis that extrinsic factors likely control the behavioral syndrome in *A. constructor*. Furthermore, soil nutrients correlated with colony personality in the field, but did not influence most colony behaviors in the greenhouse despite substantially increasing resource abundance. Soil nutrients likely interact with other environmental factors to structure the ants' behavioral syndrome in the field.

Introduction

A central aim of behavioral ecology is understanding how and why animals exhibit repeatable individual differences in behavior, or personalities (Sih et al. 2004a, 2012, Bengston et al. 2018). Recent models have generated several hypotheses about how intrinsic and extrinsic factors contribute to individual behavioral variation, plasticity, and consistency (Sih et al. 2015), suggesting that positive feedback between individual state variables and behavioral traits can contribute to stabilizing individual differences.

Social insect colonies that exhibit collective, colony-level personalities (Chapman et al. 2011, Wray et al. 2011, Pinter-Wollman et al. 2012a, 2012b, Scharf et al. 2012, Modlmeier et al. 2012, 2014a, 2014b, Bengston and Dornhaus 2014, Keiser et al. 2015, Blight et al. 2016) are excellent models to study the mechanisms controlling repeatable behavioral differences, because behavior can be easily characterized and manipulated, and colonies can be sampled repeatedly in the field due to their relatively sessile nests (Jandt et al. 2014).

We studied the factors that influence behavior in colonies of the tropical arboreal ant *Azteca constructor* that live in and protect *Cecropia* trees in return for food and nesting space (Janzen 1969, Schupp 1986). Colonies display a collective behavioral syndrome along a docile-aggressive axis under natural conditions in the field (Marting et al. 2018). Trees hosting more aggressive colonies have less leaf damage, suggesting that plant health and colony personality type may influence each other.

What causes this behavioral syndrome? Contributing factors may be intrinsic (e.g., genetics or development; Drent et al. 2003), extrinsic (e.g., environment or experience; Pinter-Wollman et al. 2012a), or a combination of both. If intrinsic factors govern colony personality, we would predict that behavioral type is independent of interactions with their hosts plants and persists long term. However, if extrinsic factors influence personality, we would predict that behavioral type may shift over time in response to environmental factors like climate, exposure to intruders, or resource availability. Higher resource availability may increase colony energy reserves, fueling higher activity and aggression. Alternatively, colonies may invest these resources in new worker production. How resources affect ant colonies may vary among *Azteca* species, several of which form associations with *Cecropia* trees. In a food supplementation field study, colonies of *A. ovaticeps* provisioned with frozen insect prey had more workers, but no such effect was seen in *A. alfari* (Dejean et al. 2012). It is possible that the nutrient boost manifested through colony behavior in *A. alfari*, but behavior was not measured in this study. Resource availability is particularly interesting in the *Azteca-Cecropia* system because host plants provide two major resources for their ant colonies – nesting space and food bodies. Soil nutrients can affect both food body production rate and nesting space

(Folgarait and Davidson 1995), thus affecting resources available to ant colonies. Soil nutrients are easy to manipulate, making them an excellent candidate to explore the effects of resource availability on colony personality.

Though it is clear that *Azteca* ants provide a benefit to their hosts by protecting them from herbivores and enriching plant nitrogen with insect prey (Janzen 1969, Schupp 1986, Sagers et al. 2000, Dejean et al. 2012, Oliveira et al. 2015), little is known about how costly they are to maintain. Plants must balance their resource allocation between growth and defense (Coley et al. 1985). To help elicit protection from ants, plants provide glycogen-rich Müllerian food bodies (Rickson 1971, Folgarait and Davidson 1995).

Allocation to food body production may detract from plant growth, which implies that ants may impose a fitness cost to the plant. In a convergent ant-plant mutualism between *Allomerus* ants and *Cordia* plants, an herbivore-by-ants factorial experiment showed that plants without their ant symbionts grew faster and produced more leaves in the absence of herbivores, suggesting that ants impose a direct cost to plant growth (Frederickson et al. 2012). By excluding herbivores, we can remove the benefit of protection and prey-based nitrogen enrichment and isolate the impact that hosting an ant colony has on plant growth. If ants do affect host plant health, the plant may shift its resource allocation which may then affect colony behavioral type, feeding back into our first major question.

We conducted a colony transplant-greenhouse fertilization experiment to test a series of hypotheses regarding colony personality, soil nutrients, and plant growth. First, we assessed the relationship between colony behavioral traits, soil nutrients, and plant growth under natural conditions in the field. Then we transplanted the colonies to *Cecropia obtusifolia* plants grown from seed in the greenhouse, left half of the

greenhouse plants without colonies, and fertilized half of the plants in a full factorial design. Finally, after 10 months of reestablishment and growth, we reassessed colony behavioral traits and measured colony size, plant growth, food body production, and leaf toughness.

Primarily, we test two competing hypotheses: 1) colony personality is driven by intrinsic colony factors; 2) colony personality is driven by extrinsic environmental factors. These hypotheses make distinct predictions about what we will see when we compare personality in the greenhouse to that originally observed in the field. If the behavioral syndrome persists through the host plant transfer, intrinsic factors like genotype likely outweigh environmental influences on colony personality. If the behavioral syndrome does not persist, extrinsic factors likely influence personality.

At the same time, we are testing a more specific hypothesis about the nature of the environmental influences on personality: the resources available to the plant affect the personality of the colony. This predicts a relationship between soil properties and colony personality in the field. It also predicts colony personality differs between the nutrient+ and nutrient- treatments in the greenhouse. By using a common garden greenhouse design, we can isolate the effects of soil nutrients on colony from other environmental interactions like local microclimate, herbivore pressure, and predation.

The hypotheses regarding the underlying mechanisms of the behavioral syndrome are not mutually exclusive. It is possible that colony behavior can persist across the transplant and also be influenced by soil nutrients, which would suggest that an interaction between intrinsic and extrinsic factors contribute to colony personality. However, if colonies neither retain their personality nor show an influence of soil

nutrients, then there are likely other environmental factors contributing to colony personality that were excluded from the greenhouse.

Finally, we test two separate hypotheses about plant growth and resource allocation: 1) ant colonies are costly to maintain; 2) plants shift defensive strategies based on their environment. By assessing the effects of soil nutrients and ants on vertical growth, food body production, and leaf toughness, we can understand how plants balance the investment in defense and growth. If ants impose a cost to *Cecropia* trees, we predict that, in the absence of herbivores, plants without colonies will grow faster than plants with colonies. If plants can shift their defensive strategies based on their environment, we predict that plants without ants will produce fewer food bodies and have tougher leaves. We also predict that the relationship between vertical growth, food body production, and leaf toughness will differ between nutrient+ and nutrient- treatments.

Methods

Study site and colony selection

We located *Azteca constructor* colonies in and around the lowland tropical rainforests of Soberania National Park, Colón, Panama between March and May 2015. Based on the correlation between tree height and colony size (see Chapter 3), we limited the variation in colony size by choosing colonies inhabiting similar-sized *Cecropia obtusifolia*, *C. peltata*, *C. longipes*, and *C. insignis* trees (height \pm SD = 4.78m \pm 1.47m, n = 18). Each tree contained a single colony, which we confirmed during the transplant

phase by locating the queen after the behavioral trials. We identified the queens using keys in Longino (2007). Queen and worker voucher specimens were deposited in the Arizona State University Natural History Collections. We identified the host *Cecropia* species using keys in Berg et al. (2005).

Colony behavioral traits

To characterize colony-level behavior, we subjected colonies to four of the same behavioral assays described in Marting et al. 2018: patrolling behavior, vibrational disturbance, response to leaf damage, and response to leafcutter ant. We added a fifth assay, response to army ant, to distinguish the colony's response to two ant intruders that pose fundamentally different threats. Leafcutter ants (*Atta colombica*) pose an indirect threat to the colony by targeting the host plant leaves (Vasconcelos and Casimiro 1997), whereas army ants (*Eciton hamatum*) pose a direct threat to colony by targeting their brood (pers. obs., Figure 4.1). Individual behavioral assays are detailed below, after we explain the overall structure of the assays. Colony behavioral traits were assessed at 3 timepoints; 1) under natural conditions in the field between June and August of 2015, 2) soon after colonies were transplanted to the greenhouse in March 2016, and 3) 10 months after transplantation to the greenhouse in July 2016. Colonies received only one assay per day. To standardize behavioral measurements across different tree sizes, we focused on the central stem at the lowest leaf's internode (henceforth, the "focal internode"), which we estimated to be the location of median colony distribution based on data from other colonies (see Chapter 3). For patrolling behavior, vibrational disturbance, and response to

intruders, we scored activity by counting the number of times we saw a worker completely traverse the lower septum line of the focal internode, regardless of direction or ant identity. For leaf damage assays, we focused on an entire leaf instead of the stem and counted the number of workers on that leaf 3 min prior to discovery. We aimed a second camcorder at the petiole-stem juncture and counted ants going to and returning from the leaf to calculate a continuous tally of ants on the leaf. Trials were recorded with HD camcorders (Panasonic HC-X900M) and scored with Solomon Coder software.

Patrolling behavior – For our measure of patrolling behaviour, we counted the absolute worker number, a distinct measure from colony response effort to a given stimulus. We recorded undisturbed, unmanipulated patrolling behavior for five minutes and scored activity as the 5-min sum of worker crossings of the focal internode.

Vibrational disturbance – We simulated a large herbivore attack by flicking the tree 10 times 1 m below the focal internode with a custom-built “flick-o-matic 2.0” that produced 10 flicks at a constant rate and force over 30 seconds. For greenhouse trees that were smaller than 1 m, we flicked the tree half the distance between the focal internode and the base of the tree. Vibrational disturbance was scored as the number of times workers crossed the focal internode during the first minute after the first flick, subtracted by the baseline (the average number of times workers crossed per minute during the three minutes immediately preceding the first flick).

Response to leaf damage – To assess the colonies’ response to leaf damage, we used a standard hole-puncher to make 6 holes in the distal tip of the largest lobe of one of

the host plants' leaves. Response to leaf damage was scored as the maximum number of workers on the damaged leaf within 10 minutes after the damage was discovered.

Response to leafcutter ant – To assess the colony's response to scouting leafcutters, we gently introduced a single *Atta colombica* worker to the focal internode. We scored response to intruder as the number of times workers crossed the focal internode during the first minute after the leafcutter made contact with the stem, subtracted by the baseline. All *A. colombica* workers were of similar size and collected from a foraging trail of a single colony.

Response to army ant – To assess the colony's response to army ants, we gently introduced a single *Eciton hamatum* worker to the focal internode with soft forceps. The ants' response was scored with the same method as for the leafcutter ant introduction. All *E. hamatum* workers were of similar size and collected from a single bivouac for each time point.

Plant growth and soil analysis from the field

To calculate plant growth rate, we measured tree height and diameter at 2 time points in the field; March and September 2015. We collected soil samples from the base of the trees in the field to test the natural relationship between soil nutrients, plant growth, and colony behavior. Using a soil core, we collected the top 10 cm of soil from 5 equidistant points along a circle of 1 m radius centered at the base of each tree. The samples were combined and homogenized to create 1 soil sample per tree and

immediately returned to the laboratory for standard analysis of resin phosphorus, total inorganic nitrogen, and extractable potassium (see Turner and Romero 2009 for details).

Greenhouse preparation and maintenance

We chose to use *Cecropia obtusifolia* for the greenhouse experiment because they occur naturally in both rich and poor soil types across Panama (Condit et al. 2013, Ben Turner pers. com.) and are the most common *Cecropia* species associated with *Azteca constructor* (Marting et al. 2018). We wanted to limit between-plant variation in the greenhouse and therefore collected fruits from the base of a single mature tree along Pipeline Road in Soberania National Park, Colón, Panama on March 5, 2015. With running water, we dislodged the seeds from the fruit, drained the pulp, and isolated the seeds. We immediately sterilized them by rinsing them in 95% ethanol for 10 seconds, 10% bleach for 2 minutes, then 70% ethanol for 2 minutes. We germinated the seeds in a seedling tray with Miracle-Gro Continuous Release Potting Mix. On April 13, 2015, we transplanted seedling to 20-liter pots that contained a homogenized mixture of 50% river sand and 50% nutrient-poor soil excavated near Colón, Panama. The greenhouse in Gamboa, Panama was 8m by 12m, in full sun, and was enclosed in insect-prevention screening that transmitted 95% sunlight. Plants were watered three times per day and rotated positions every month. Once the saplings began to grow hollow internodes and food bodies (~6 months after germination), they were ready to receive colonies.

Colony transplants and nutrient treatments

On the day before transplanting colonies from the field to the greenhouse, we prepared their target greenhouse plant by opening holes in the 3 most apical prostomata with the punch blade of a pocket knife to pre-form entrances into the internodes. Pilot studies showed that pre-formed entrances increase the likelihood of colonization and decrease immigration time. Additionally, *Cecropia* saplings occasionally flood the internodes with fluid after the prostomata have been punctured, which prevents any insects (including *Azteca* ants) from entering. The flooding typically subsides after 24 hrs, so preparing the internodes a day in advance was sufficient.

To collect the entire colony, we cut the trees at the base with a machete over a large tarp, separated the leaves, cut the stem into meter-long segments, and placed all the plant material into a large plastic bag. We immediately transported the bags from the field to a pavilion near the greenhouse and spread all the plant material on another large tarp. With a machete and mallet, we split open each stem segment to access the nest. We collected the queen and stored her in a vial for safe transfer.

Based on preliminary transfer attempts, we decided that 500 workers were sufficient for colony reestablishment, with an additional buffer of 30 workers to account for casualties during the transplant. Using aspirators, we collected workers from every surface (leaves, tarp, external stem, inside different internodes, etc.) to generate a uniform representation of worker demographics. We incrementally transferred collected ants to a Tupperware container, photographed them, and quickly counted them using ImageJ on a laptop until we had collected a total of 530 live workers. We calculated the average

proportion of workers-to-brood, -scale insects, and -refuse piles using sociometry data from other colonies (see Chapter 3) and scaled them down to 500 workers for the transplant. We collected the following nest components from the internodes of each colony to include in the transfer: 120 brood (mixed instar), 10 scale insects, and 4 refuse piles.

From separate containers, we placed the queen, workers, brood, scale insects, and refuse piles on the surfaces of the leaves and the soil in the base of the pot. Pots stood on a 15 cm PVC platform inside a large aluminum baking tin full of water that acted as a mote to prevent escape and invasion. Within 10min, workers recruited to the entrances, began collecting the brood, and guided the queen into one of the opened internodes. Each colony received a small vial of sugar water and a cricket to ease them through the transplant. No additional supplements were provided to the colony for the remainder of the experiment.

All colony transplants occurred in September 2015. Half of the plants in the greenhouse received ant colonies (n=22/41) and half received soil nutrients (n=20/41) in a full factorial design (Figure 4.2). Soil nutrient treatments began on October 1, 2015, a week after colony transplants were complete. Plants in the (+) nutrient treatment received a monthly dose of 5g of general, slow-release fertilizer for 6 months (Osmocote Smart-Release Flower and Vegetable Plant Food 14-14-14; 14% total nitrogen (ammoniacal nitrogen, nitrate nitrogen), 14% available phosphate, and 14% soluble potassium)). Plants in the (-) nutrient treatment received no fertilizer. The only colonies that died completely after the transplant (n=5) did so in the first 2 weeks and their plants were thus treated as if they did not receive colonies in further analysis. Colonies that were not *Azteca*

constructor were excluded from further analysis (*A. isthmica*, n=2; *A. alfari*, n=1).

Therefore, the effective number of trees in each treatment were as follows: A-N-, n=11; A+N-, n=8; A-N+ n= 11; and A+N+, n=6 (where - = absent, + = present, A = ants, and N = nutrients).

Food body production

After the final behavioral trials were complete in July 2016, we measured Müllerian food body production rates. We removed all visible food bodies from each plant and immediately wrapped each trichilium with 4 layers of Parafilm to prevent ant access. After 24h, we removed the Parafilm and used a tally meter to count the food bodies that had grown.

Greenhouse harvest

We harvested all the plants in the greenhouse in August 2016 to measure plant and colony growth. We measured plant height, diameter, number of leaves, and number of internodes at 3 time points; 1) when colonies were transplanted in September 2015, 2) during the early behavioral assessment in March 2016, and upon harvesting the plants in August 2016. We removed all leaves from the plant and immediately measured leaf toughness by piercing 2 center points and 2 distal lobe points of each leaf using a penetrometer. We calculated a leaf toughness score for each plant by averaging the measurements across piercing points for all leaves.

To collect the colonies, we subdued them using a topical insecticide spray (active ingredients: pyrethrins, piperonyl butoxide, and permethrin), split open the stems over a large tarp, and collected all the workers and the queen in a large vial with 95% ethanol. To survey colony size, workers were spread out on grid paper, photographed, and counted using ImageJ software.

Statistical analyses

Colony consistency for each behavioral trait was analyzed by separately regressing the scores from the first trial in the field to the first trial in the greenhouse, the first trial in the field to the second trial in the greenhouse, and the first greenhouse trial to the second greenhouse trial. At each time point, relationships among behavioral traits were analyzed by entering the average scores for significantly consistent traits into an unrotated principal component analysis (PCA). To select which components to retain, we used the Kaiser-Guttman stopping rule, which drops components with eigenvalues less than the mean eigenvalue (Jackson 1993). We assessed the consistency of colony personality by regressing PC1 from the field with PC1 from the second greenhouse. We assessed the effect of time point on colony behavior at the population level with a repeated measures ANOVA for each behavioral trait.

To assess the correlation between soil nutrients, plant growth, and colony personality in the field, we separately tested for linear correlation of each nutrient (N, P, K, and N:P ratio) with plant growth rate and colony personality (PC1). We evaluated the effects of soil nutrient treatment with a t-test for plant growth, food body production,

estimated plant volume, and colony size (number of workers). To evaluate the effects of soil nutrients on colony behavior, we calculated the change in behavior by subtracting the colony scores from the field by the colony scores from the second greenhouse assessment separately for each behavioral trait. We then compared the mean change in behavior between nutrient treatments with a t-test. We also compared the mean behavioral score on PC1 between nutrient treatments with a t-test.

To analyze how the environment affects plant investment, we used a general linear model (GLM) with nutrients, ants, and their interaction as factors for plant growth, food body production, and leaf toughness. To determine the relationship among these factors, we ran PCAs including plant growth, food body production, and leaf toughness as factors separately for each nutrient treatment.

Results

Colony behavior from the field to the greenhouse

At the population level, the magnitude and variation of behavioral traits did not differ between the field assessment and the second greenhouse assessment overall (except for response to leaf damage, which was higher in the greenhouse; paired t-test, $p = 0.002$). However, both magnitude and variation were reduced in the first greenhouse assessment (Figure 4.3). We therefore considered colonies to be still reestablishing during the first greenhouse assessment and did not analyze these data in depth.

Under natural field conditions, colonies differed substantially in their response to all assays (range of colony responses for patrolling: 5 – 530 crossings; disturbance: 4 – 492 crossings; leaf damage: 16 – 313 ants; leafcutter ant intruder: -21 – 331 crossings; army ant intruder: -5 – 608 crossings). A principal component analysis including all 5 colony traits showed that the first component (PC1) explains 59.1% of the variation with an eigenvalue of 2.96 and that all the traits loaded strongly positive (Figure 4.4a, Table 1). The second component's eigenvalue was less than the mean (0.89) and was therefore excluded from further analysis (Jackson 1993). We used colony scores on PC1 to characterize each colony's behavioral type along an aggressive-docile axis, with higher scores indicating greater activity, aggression, and responsiveness. Colony behavioral type was not correlated with tree height, estimated colony size (correlation, $n = 14$, $p = 0.84$, range 4,419 – 11,691), or estimated age (correlation, $n = 14$, $p = 0.30$, range: 1.3 – 4.0 years). The pattern and relationship among behavioral traits found here closely resembled those found in an earlier study (Chapter 2, Marting et al. 2018)

Under greenhouse conditions, colonies differed substantially from one another in their response to all assays (range of colony responses for patrolling: 32 – 979 crossings; disturbance: 13 – 519 crossings; leaf damage: 96 – 419 ants; leafcutter ant intruder: 8 – 187 crossings; army ant intruder: 23 – 230 crossings). A principal component analysis including all 5 colony traits showed that traits were related in a complex way that was difficult to interpret (Table 2, Figure 4.4b). The first 3 principal components had eigenvalues greater than the mean, indicating that they all capture important variation, but loading scores overlapped and interacted. PC1 primarily separates response to leaf damage from other behaviors, PC2 is mostly dominated by response to vibrational

disturbance, and PC3 separates patrolling activity and response to army ants. No colony behavioral traits correlated with plant height or colony size.

Although some colonies behaved consistently for some behavioral traits, no colony behaved consistently for all traits (see Appendix E for examples). Overall, colony behavioral traits were not statistically consistent across the transplant; behavior in the greenhouse was not correlated to behavior in the field (patrolling: correlation, $p = 0.48$; disturbance: correlation, $p = 0.97$; leaf damage: correlation, $p = 0.96$; leafcutter: correlation, $p = 0.85$; army ant: correlation, $p = 0.75$).

The effect of soil nutrients on plant growth and colony behavior

In the field, host plant growth rate was negatively correlated with the soil N:P ratio (Figure 4.5, correlation, $n = 17$, $p = 0.009$), but was not correlated to any nutrient by itself (correlation, total inorganic nitrogen: $n=18$, $p = 0.45$; potassium: $n=18$, $p = 0.56$; phosphorus: $n=17$, $p = 0.28$). Colony behavioral type was positively correlated with soil phosphorus (Figure 4.6, correlation, $n = 13$, $p = 0.01$) and potassium (Figure 4.7, correlation, $n = 14$, $p = 0.0002$), but was not correlated to total inorganic nitrogen (correlation, $n = 14$, $p = 0.40$) or N:P ratio (correlation, $n = 13$, $p = 0.80$).

In the greenhouse, plants that received soil nutrients grew 2.4x taller (t-test, $p < 0.0001$, Figure 4.8A), increased 7.9x more in estimated volume (t-test, $p < 0.0001$, Figure 4.8B), and produced 2.5x more food bodies (t-test, $p < 0.0001$, Figure 4.8C) than plants that did not receive nutrients, resulting in colonies with 4.1x as many workers (t-test, $p < 0.001$, Figure 4.8D).

Soil nutrients did not have a significant effect on any colony behavior except patrolling, for which colonies that received soil nutrients had an increase in patrolling activity (t-test, $p = 0.01$, Figure 4.9).

Ant presence and plant investment

For plant vertical growth, soil nutrient treatment and its interaction with ant presence had a significant effect, but not ant presence alone (GLM: nutrient effect, $p < 0.0001$; ant effect, $p = 0.82$; interaction effect, $p = 0.026$; Figure 4.10). Plants that received soil nutrients grew taller if they had an ant colony (t-test, $p = 0.038$). For food body production, both soil nutrients and ant presence had a significantly positive effect, but not their interaction (GLM: nutrient effect, $p < 0.0001$; ant effect, $p = 0.040$; interaction effect, $p = 0.23$; Figure 4.11). Plants that did not receive soil nutrients produced more food bodies if they had an ant colony (t-test, $p = 0.011$). For leaf toughness, only soil nutrients had a significantly positive effect (GLM: nutrient effect, $p < 0.0001$; ant effect, $p = 0.36$; interaction effect, $p = 0.27$).

We ran a PCA including vertical growth, food body production, and leaf toughness separately for both nutrient treatments to determine how resource abundance affects plant investment strategies. The PCA structure depended on the nutrient treatment, and only the first principal component was significant for both PCAs (Table 3). For plants without nutrients, food body production and leaf toughness loaded strongly positive while vertical growth loaded strongly negative on the first principal component. We interpret PC1 as “defense investment” because high values indicate investment in

food bodies and leaf toughness over vertical growth. For plants with nutrients, food body production and vertical growth loaded strongly positive while leaf toughness loaded strongly negative on the first principal component. We interpret PC1 as “production investment” because high values indicate investment in food body production and vertical growth over leaf toughness. The presence of ants did not have a significant effect on plant investment strategies.

Discussion

Our results strengthen the evidence that colonies of *Azteca constructor* maintain a collective behavioral syndrome along a docile-aggressive axis under natural field conditions, but we show that the structure of this behavioral syndrome unravels when colonies are transplanted to the greenhouse. Colonies did not retain their personalities in the greenhouse, which supports the hypothesis that extrinsic environmental factors outweigh intrinsic colony factors influencing colony personality in *A. constructor*. Furthermore, soil nutrients correlated with colony personality in the field, but did not influence most colony behaviors in the greenhouse despite substantially increasing resource abundance. Soil nutrients likely interact with other environmental factors to structure the behavioral syndrome in the field.

Behavioral traits in the greenhouse were not related with one another in the same way they were in the field. In the field, the behavioral syndrome was structured with all traits related in the same direction, which is remarkably similar to the syndrome described in the study conducted two years earlier with different colonies (Marting et al.

2018). This underscores that the behavioral syndrome is a robust phenomenon and possibly under stabilizing selection in the field. However, in the greenhouse, traits were unrelated, suggesting that the factors that tether behavioral traits were excluded from the greenhouse. Perhaps in the greenhouse, devoid of regular encounters with herbivores and intruders, behavioral responses began to drift with the emergence of new, unexperienced workers, and behavioral traits ultimately became decoupled. Correlations among traits may depend on behavior-state feedback loops involving herbivores (Sih et al. 2015). For example, colonies with higher energy reserves may be able to capture more insect prey, not only increasing their energy stores, but reducing herbivory for their host plant which in turn produces more food bodies. This increased energy may then fuel more aggressive behavior.

Even though colonies did not respond to behavioral assays consistently from the field to the greenhouse, at the population level, the variation and mean scores of most behavioral traits did not differ between locations for most traits. This demonstrates that as a population, the greenhouse did not change the way colonies responded to behavioral assays. The exception is that colonies responded stronger to leaf damage in the greenhouse, which may reflect their motivation to capture prey. This makes sense given that the greenhouse likely afforded fewer opportunities to encounter insect prey than the field. Perhaps their increased response indicates that colonies were protein-starved.

Although there is extreme local heterogeneity in soil fertility (Townsend et al. 2007, Condit et al. 2013), global patterns demonstrate that tropical ecosystems are nitrogen-rich and phosphorus-poor (Hedin 2004, Reich and Oleksyn 2004, Turner et al. 2018), leading to the prediction that high P soil promotes plant growth. For *Cecropia*

trees in the field, we show that no soil nutrient alone correlated with growth rate, but trees in soil that had more phosphorus *relative* to nitrogen (N:P ratio) grew faster, suggesting that plants are P-limited. This limitation may be driven by the fact that plants get a substantial amount of nitrogen from their *Azteca* ants via insect prey (Sagers et al. 2000, Dejean et al. 2012, Oliveira et al. 2015).

Colony personality correlated with soil phosphorus and potassium in the field, but not nitrogen or N:P ratio. It is possible that both relationships may be driven by two outliers, in which case soil and personality may be unrelated. However, these high nutrient, high aggression points may be driven by a threshold effect. One possible pathway to this trend is that the soil nutrients that the plant cannot use for vertical growth somehow instead influence the nutrient ratio of the food bodies they provide to the ants. The shift in food body nutrients may in turn promote higher colony energy stores or metabolism, which may manifest as increased colony aggression. Another possibility is that these soil nutrients enrich the nutrients of the plant leaves, which often increases herbivory pressure (Mendes and Cornelissen 2017). An increased encounter rate with insect herbivores may lead to sensitization over time and increased colony aggression.

In the greenhouse, soil nutrients increased both plant and colony growth, but did not influence most colony behaviors except for patrolling activity. Instead of increasing aggression with the increased resources, colonies invested in more workers. Perhaps the fact that the fertilizer contained equal parts N:P:K has something to do with this pattern. Given the pattern from the field that plants grow fast when N:P ratio is lower, and colony aggression only correlated with soil P and K alone, perhaps manipulating N, P, and K

separately would elucidate the relationship between nutrients, plant growth and colony aggression.

We offer a speculative explanation for patterns of soil nutrients, plant growth, and colony personality from the field to the greenhouse. Trees use soil nutrients to increase vertical growth, which increases colony growth. The excess nutrients that plants cannot use end up in the food bodies and leaves, shifting the nutrient content offered to the ants and increasing colony encounter rate with insect herbivores. The interaction between increased nutrients and experience with intruders increases colony aggression, which increases prey capture, stabilizing colony personality. We saw a disassociation between behavioral traits and their relationship with soil nutrients because herbivores were excluded from the greenhouse. To test this hypothesis, we suggest a factorial experiment using different soil nutrients and levels of herbivore exposure.

Our results show that plants without ants did not grow faster than plants with ants, suggesting that *A. constructor* is not costly to maintain for *C. obtusifolia*. In fact, plants that received nutrients saw an increase in growth when hosting an ant colony. The greenhouse was competent at excluding insects, but perhaps not perfect. It is possible that a very small amount of prey landed on the plants and ant colonies captured and deposited extra nutrients to their plants. Perhaps plants without nutrients did not receive the same benefit from the ants because the colonies were too small to make a difference. Ant colonies may provide other services to the plants: meticulous cleaning by removing bits of debris may increase photosynthetic potential or prevent fungal and bacterial pathogens from taking hold. This result provides an interesting contrast to the *Allomerus-Cordia* ant-plant mutualism, where plants incur a substantial reduction in growth rate by hosting

an ant colony (Frederickson et al. 2012). The benefits of hosting an ant colony for *Cordia* depend heavily on context, but *Cecropia* plants have nothing to lose and everything to gain. However, there may be costs under natural conditions not measured in this study, such as attracting a destructive anteater attack (see Appendix F).

Only plants that did not receive nutrients produced more food bodies when hosting a colony. Colonies may stimulate higher rates of food body production (Folgarait and Davidson 1995), but we did not detect increased food body production by plants with colonies under high nutrients. This suggests that plants with limited resources may reduce their investment in food bodies.

Under high soil nutrients, plants had higher vertical growth, food body production, and leaf toughness, suggesting that nutrients increase plant performance overall. Additionally, the relationship among vertical growth, food body production, and leaf toughness differed between nutrient treatments. Plants without nutrients show a tradeoff between defense traits and growth, while plants with nutrients show a tradeoff between leaf toughness and new growth (vertical and food body). Taken together with the interactions of ant presence and soil nutrients discussed above, this provides strong evidence that plants shift their defensive strategies based on their environment.

Our study suggests that colony personality is primarily shaped by extrinsic, environmental factors given that behavioral consistency and the relationship among traits disappears when colonies are removed from the field. Furthermore, soil nutrients may only be an important determinant of personality in the presence of other environmental factors like exposure to herbivores and predators. Although we did not detect the effects of intrinsic colony factors on colony personality, they may still play a role. Perhaps

factors like genotype do not directly correlate with aggression, but correlate with traits that influence aggression, such as sensitization, recruitment behavior, and behavioral flexibility. Revealing such factors may require long term field experiments that assess the heritability and relative success of behavioral traits (Gordon 2013).

Figures and Tables



Figure 4.1. The army ant *Eciton hamatum* successfully raiding an *Azteca constructor* colony.

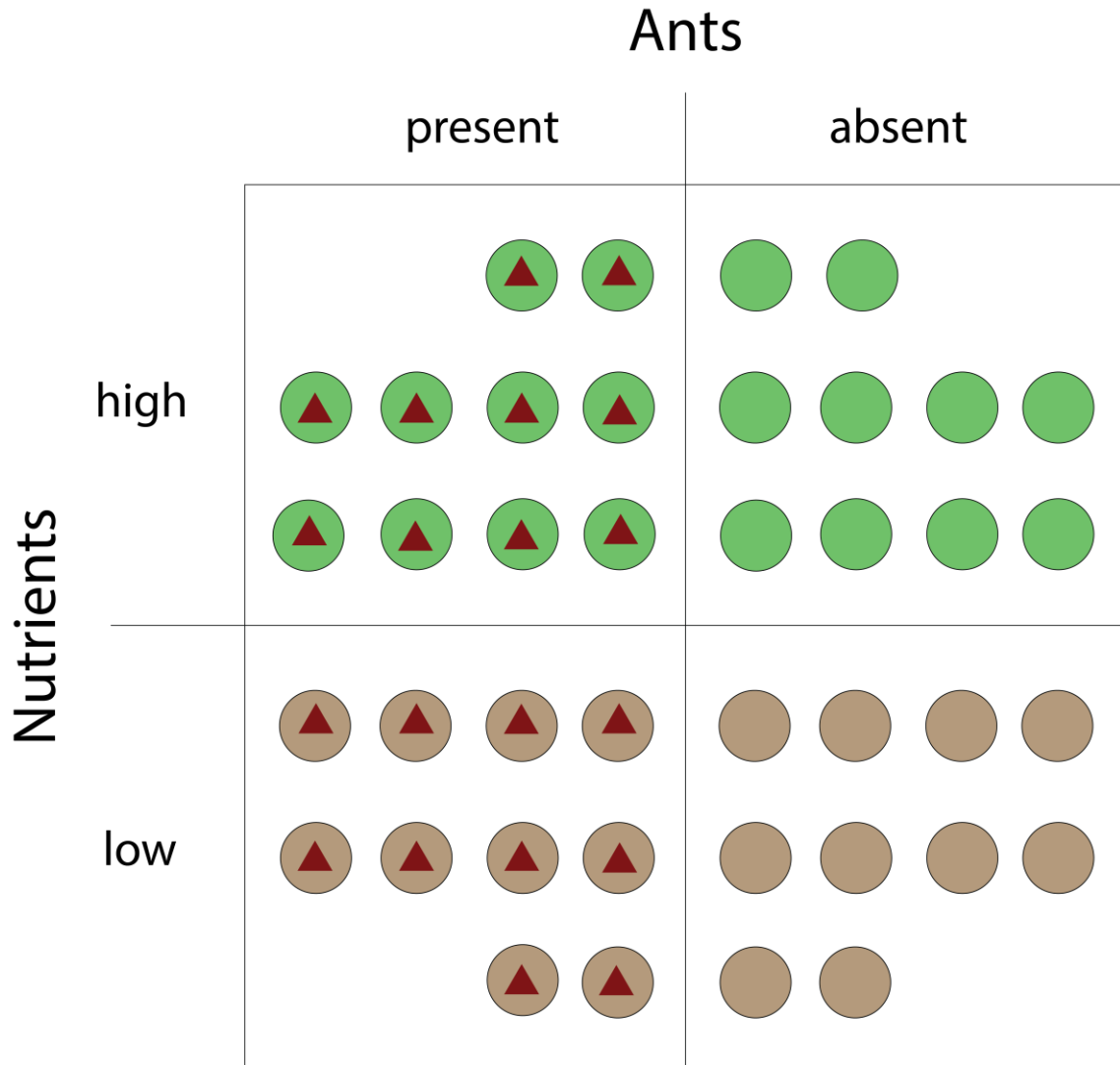


Figure 4.2. Diagram of the greenhouse experimental design.

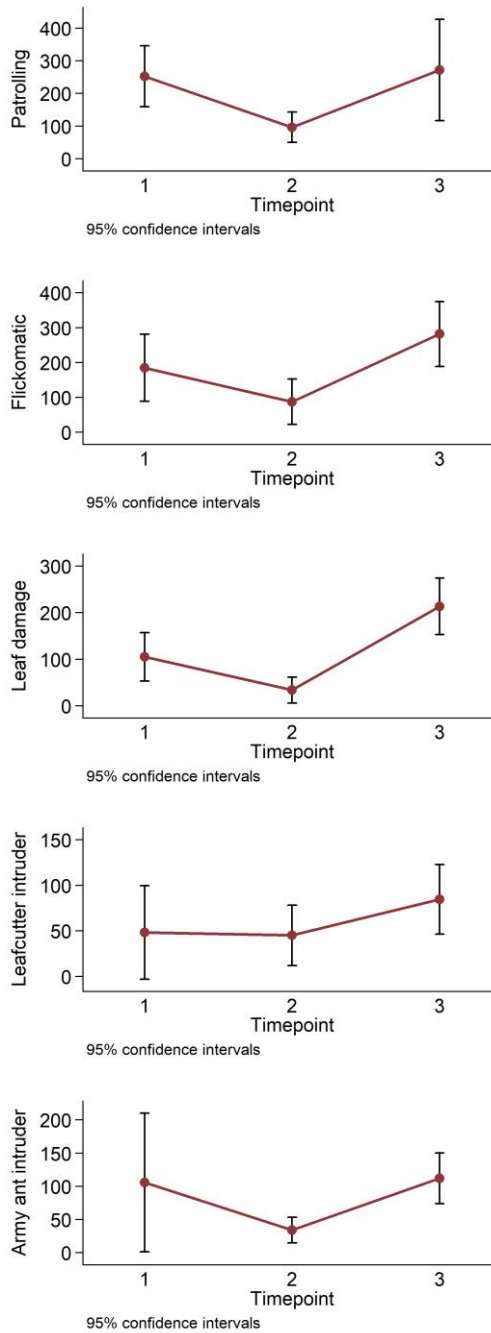


Figure 4.3. Behavioral traits across time at the population level. Points represent means and error bars represent 95% confidence intervals. Timepoint 1 is in the field, 2 is shortly after transplant to the greenhouse, and 3 is 10 months after the greenhouse transplant.

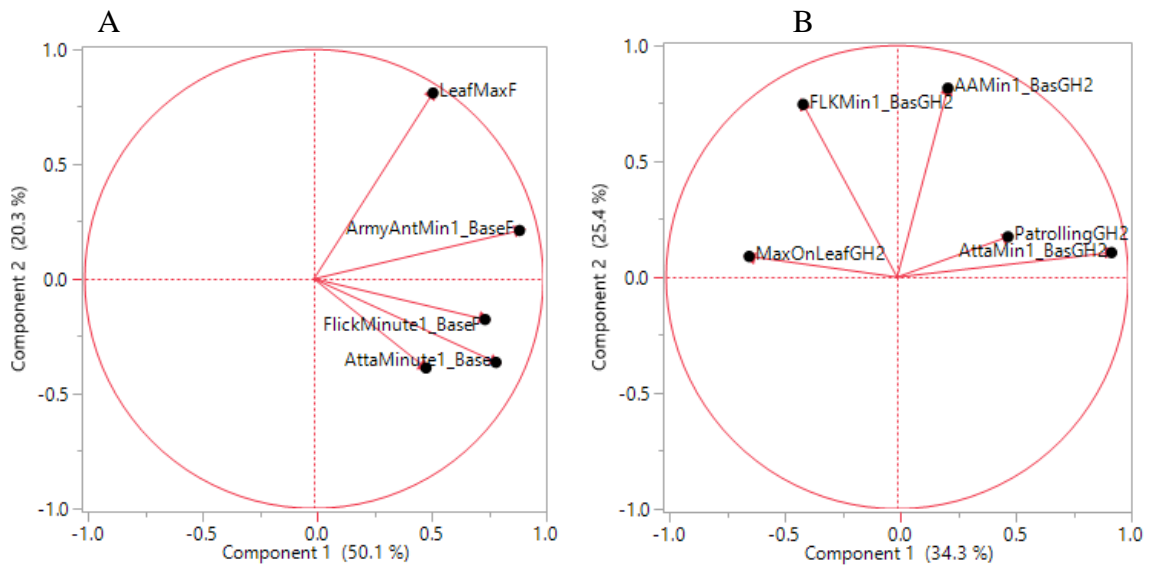


Figure 4.4. Principal components loading scores for behavioral traits A. in the field and B. in the greenhouse 10 months after the transplant.

Table 4.1. A summary of the principal component analyses for behavioral traits in the field. Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	2.983	0.860
Variance Explained	59.7%	17.2%
Loading Scores		
Patrolling	0.3885	-
Vibrational disturbance	0.4673	-0.2357
Leaf damage	0.3407	0.8372
Leafcutter ant	0.4806	-0.4831
Army ant	0.5327	-

Table 4.2. A summary of the principal component analyses for behavioral traits in the greenhouse. Dashes indicate loading scores below 0.2.

	PC1	PC2	PC2
Eigenvalue	1.626	1.276	1.073
Variance Explained	32.5%	22.5%	21.5%
Loading Scores			
Patrolling	0.3847	0.3781	0.6844
Vibrational disturbance	-	0.7115	-
Leaf damage	-0.4786	0.4128	0.3124
Leafcutter ant	0.7192	-	-
Army ant	0.3035	0.4245	-0.6315

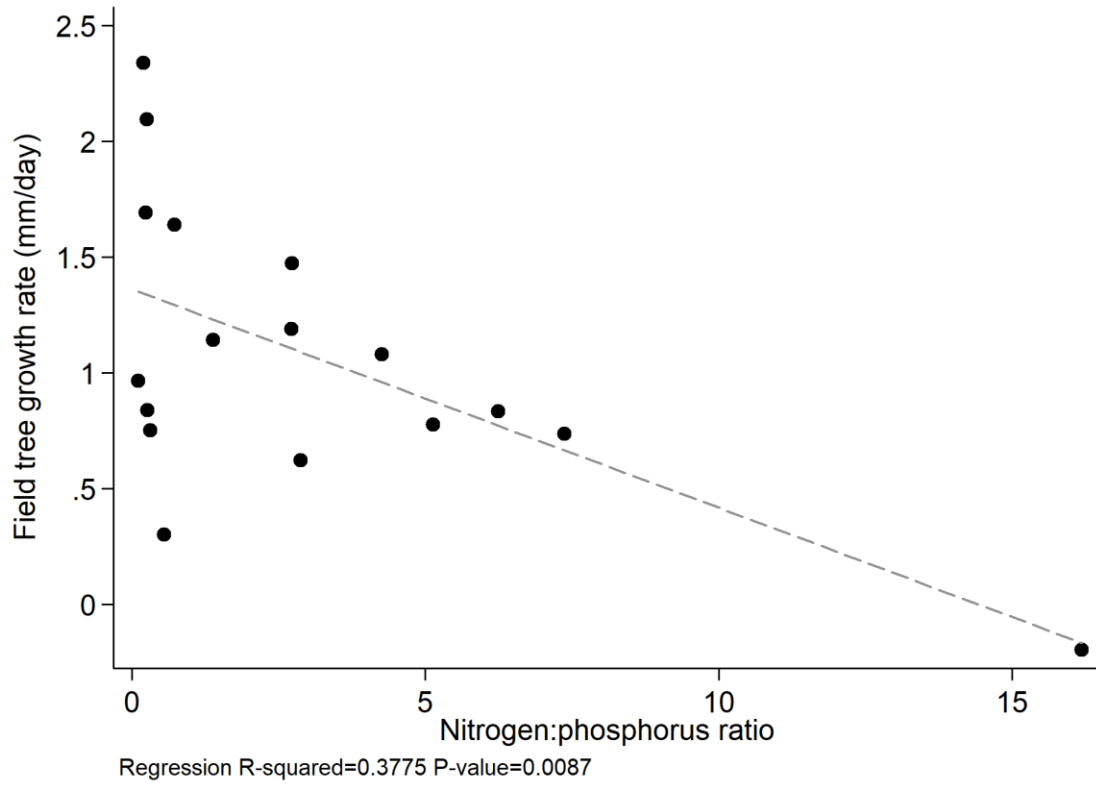


Figure 4.5. The relationship between tree growth rates and soil N:P ratio in the field. The dashed line represents a linear regression.

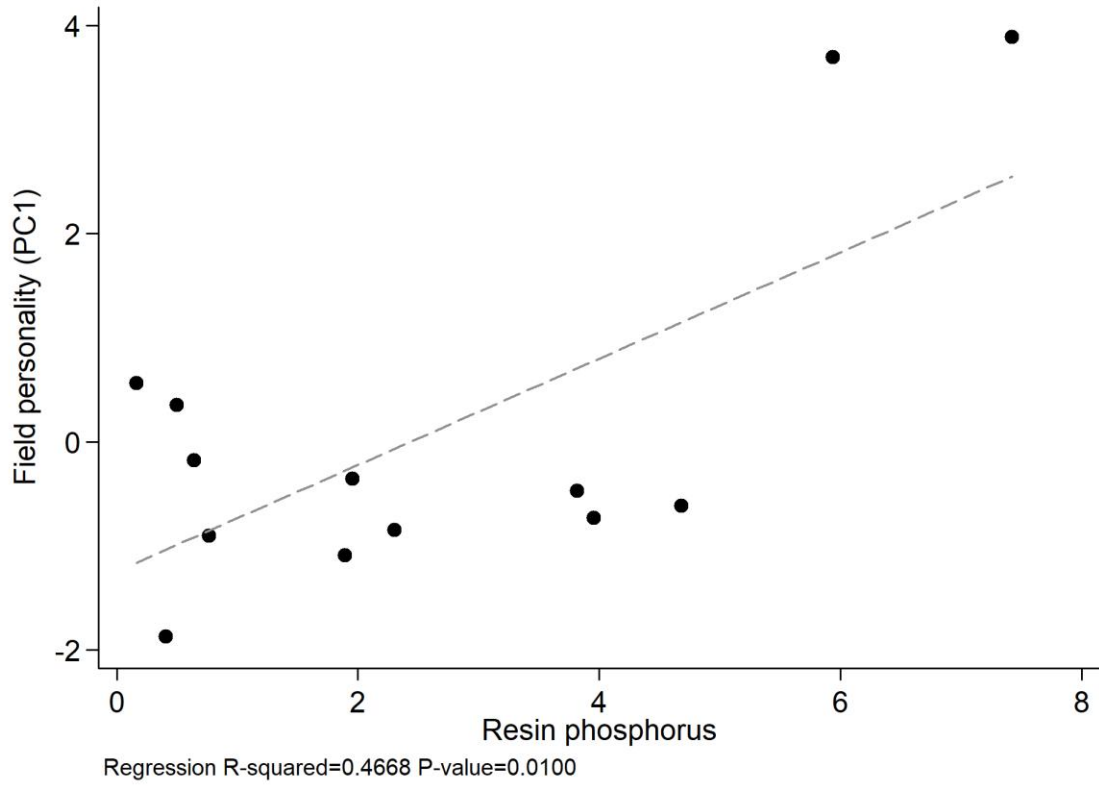


Figure 4.6. The relationship between colony personality and soil phosphorus in the field.

The dashed line represents a linear regression.

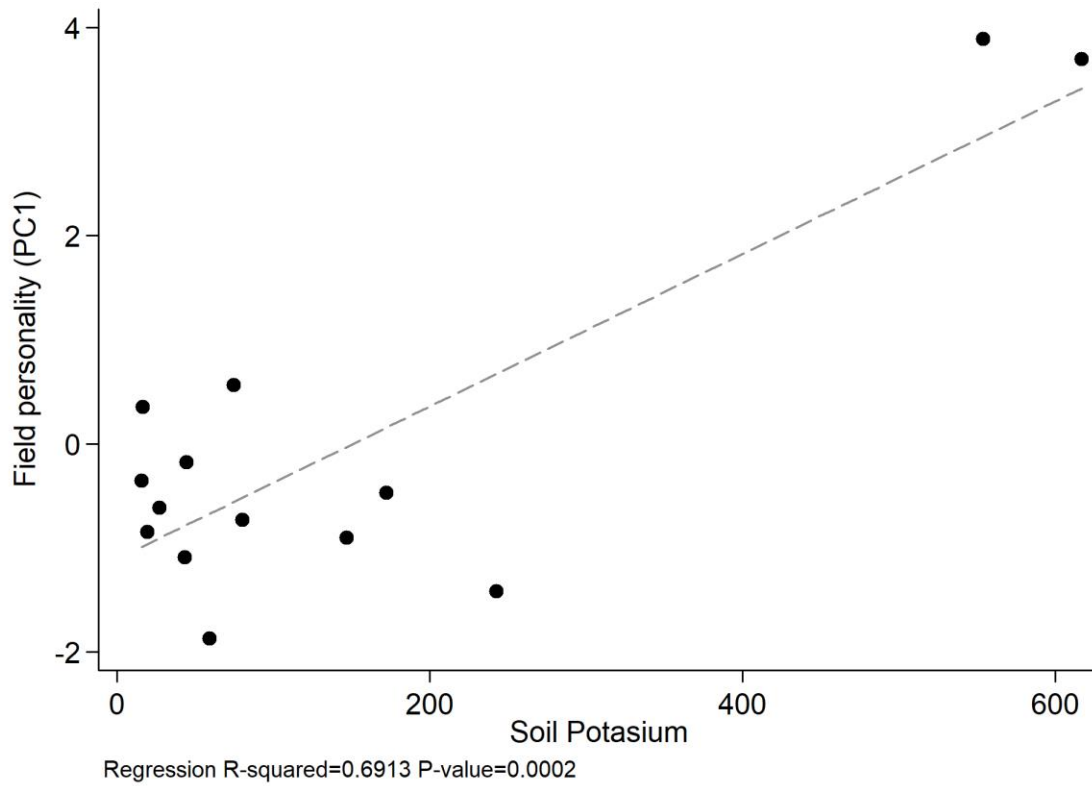


Figure 4.7. The relationship between colony personality and soil potassium in the field.

The dashed line represents a linear regression.

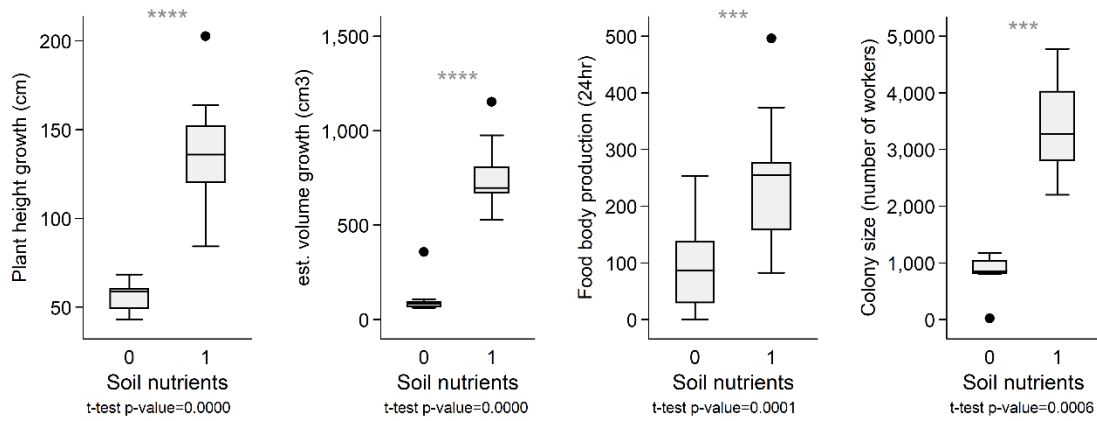


Figure 4.8. The effect of soil nutrient treatment on plant and colony growth after 10 months. A. Plant vertical growth (change in height from the beginning of the colony transplants and nutrient introductions). B. Estimated volume growth. C. Food body production rate (food bodies per day). D. Colony size as measured by total number of workers.

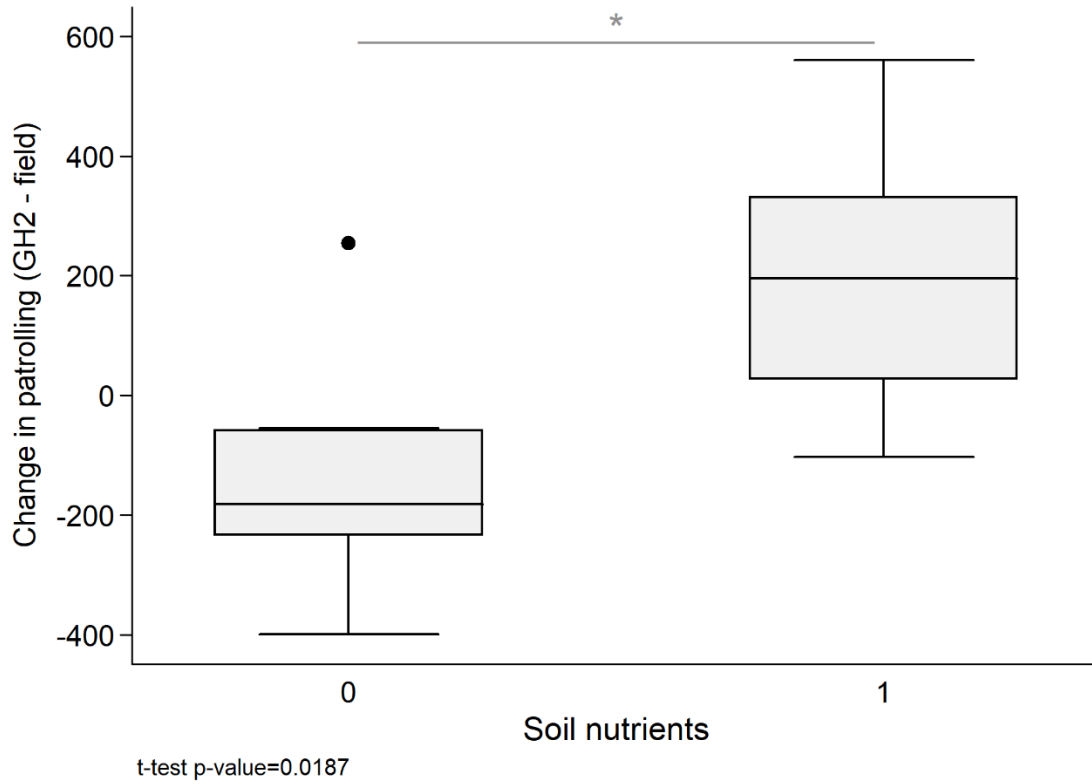


Figure 4.9. The effect of nutrient treatment on patrolling behavior. Change in patrolling was calculated by subtracting colony scores in the greenhouse from colony scores in the field.

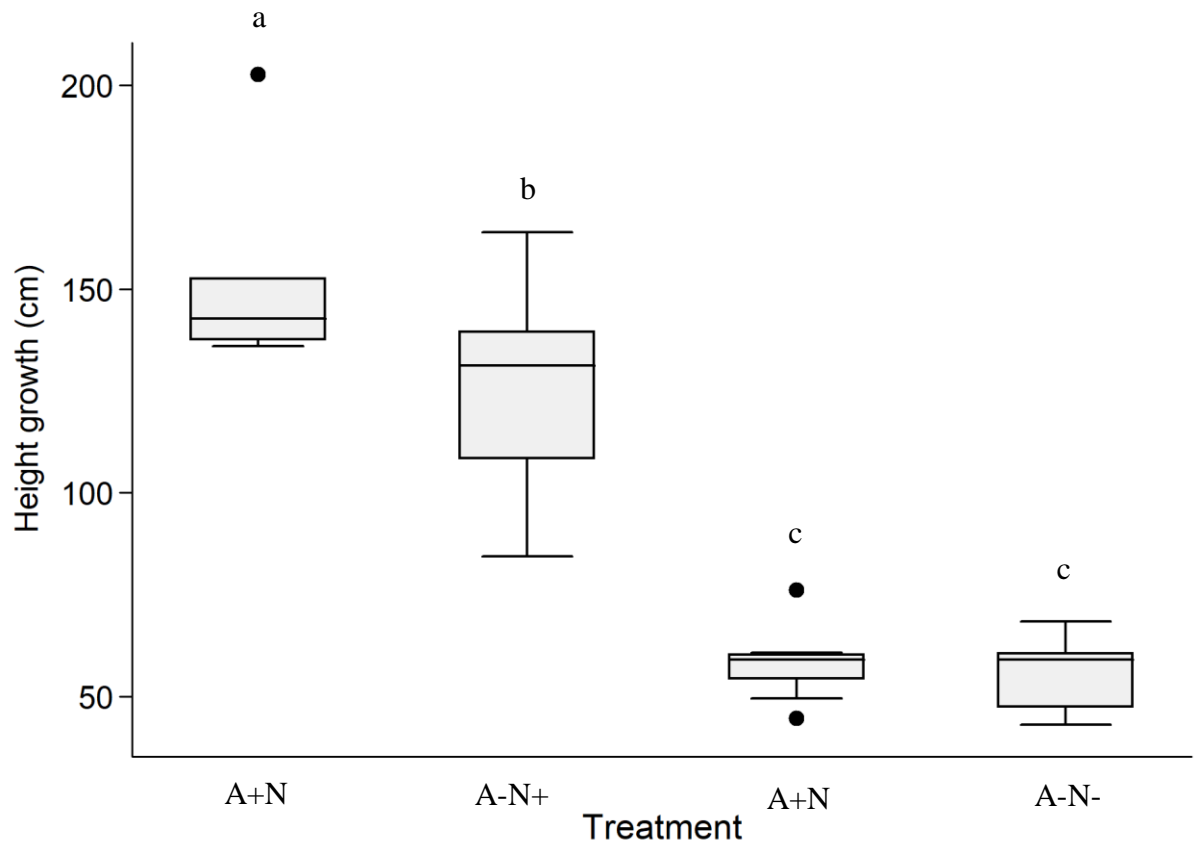


Figure 4.10. The effect of ants and nutrients on plant growth. Treatment codes are as follows: - = absent, + = present, A = ants, and N = nutrients.

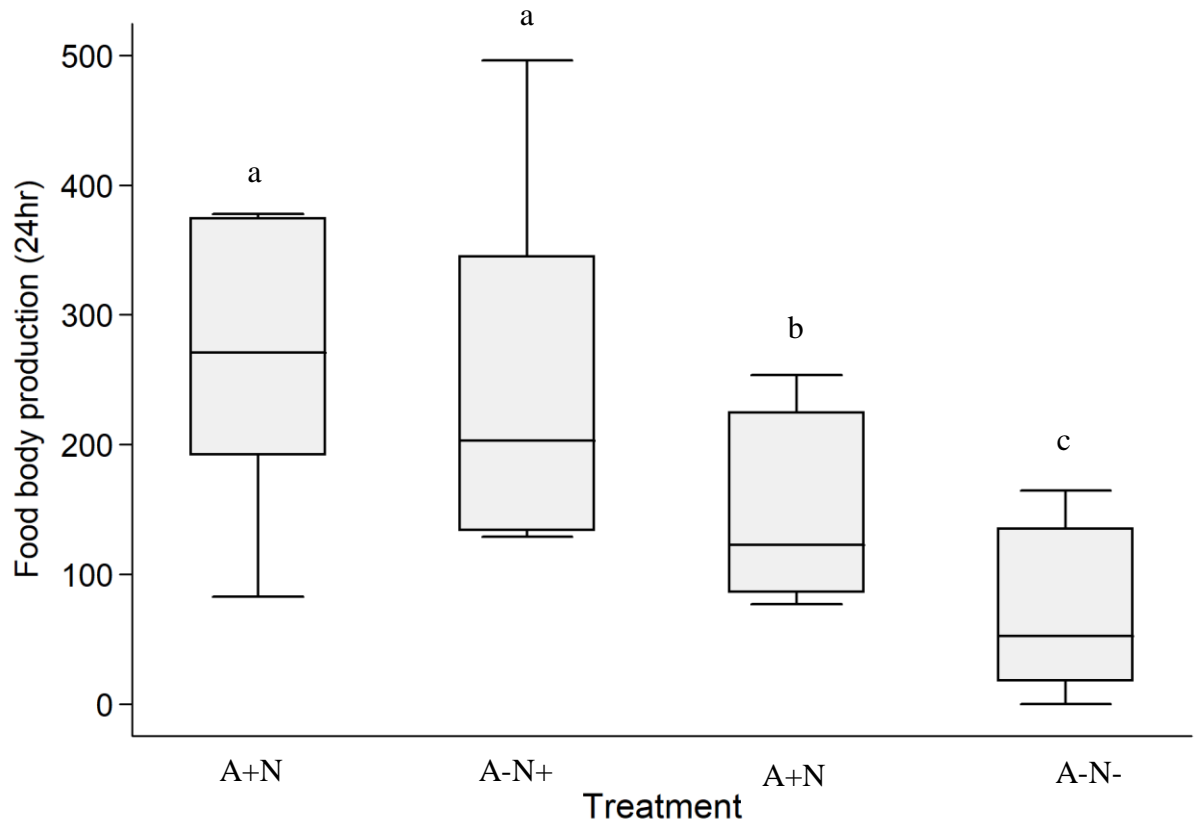


Figure 4.11. The effect of ants and nutrients on food body production. Treatment codes are as follows: - = absent, + = present, A = ants, and N = nutrients.

Table 4.3. A summary of the principal component analyses for plant investment for plants that did not receive soil nutrients. Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	1.623	0.835
Variance Explained	54.1%	27.8%
Loading Scores		
Height growth	-0.5059	0.7866
Food body production rate	0.5674	0.6126
Mean leaf toughness	0.6497	-

Table 4.4. A summary of the principal component analyses for plant investment for plants that received soil nutrients. Dashes indicate loading scores below 0.2.

	PC1	PC2
Eigenvalue	1.370	0.975
Variance Explained	45.7%	32.5%
Loading Scores		
Height growth	0.4770	0.7549
Food body production rate	0.6945	-
Mean leaf toughness	-0.5386	0.6557

CHAPTER 5

CONCLUSIONS

Summary

This dissertation furthers our understanding of the fascinating behavioral ecology of the *Azteca-Cecropia* mutualism. I demonstrate that colonies display collective personalities in the field that correlate with host plant leaf damage. Consistency and correlation among behavioral traits disappear under controlled greenhouse conditions, suggesting that extrinsic environmental factors in the field are important for structuring the collective behavioral syndrome. Herbivores may play an important role since they were excluded from the greenhouse. While I show that leaf damage and soil nutrients correlate with colony personality in the field, I have yet to measure all three in the same study. It would be interesting to elucidate how these factors interact through soil nutrients by herbivory factorial experiment.

The hypothesis put forth in Chapter 3 that colony and plant growth rates are aligned was further supported by the results from the greenhouse experiment in Chapter 4: plants that grew faster hosted colonies that grew faster. Furthermore, ants did not appear to impose a cost for their host plants, but instead provided a growth boost under favorable nutrient conditions in the absence of herbivory. Taken together with the evidence that more aggressive colonies live in plants with less leaf damage from Chapter 2, it is likely that the *Azteca-Cecropia* mutualism is under stabilizing selection through positive feedback loops: what is good for the ants is good for the plants and vice versa.

I described in detail for the first time how *Azteca* ant colonies are distributed and organized throughout their host plant. I showed that colonies ranged in their vertical distributions from low and broad, to high and narrow. How colonies distributed themselves were independent of their collective personality, suggesting that separate factors drive colony variation on these two axes.

As with any intriguing study system, this exploration of the *Azteca-Cecropia* mutualism has left me with far more questions than I have answers. I am excited to base my career on these questions moving forward. In the next section I outline specific projects that I have started and aim to complete, all stemming from this dissertation research.

Future Directions

Exposure to herbivores – Experience can influence animal personality traits (Frost et al. 2007). The effects of experience may be particularly pronounced for *Azteca* colonies, which are bound to their local microhabitat through their tree, and therefore likely to accrue certain experiences at different rates, such as herbivory (Coley and Barone 1996). I hypothesize that prolonged exposure to herbivores influences colony personality. To test this, I plan to raise colonies in trees in the greenhouse and divided them into treatments that are exposed to different densities of grasshoppers to simulate different herbivory pressures.

Energetic costs of aggression – In order for behavioral syndromes to be maintained in a population, there are likely trade-offs between different behavioral types

– certain personalities should excel in some situations and fall behind in others (Sih et al. 2004a). For *Azteca* colonies, the benefits of being a more active, aggressive colony are easily observed in their effectiveness at protecting the colony and their tree. But what about the costs? A likely candidate is higher metabolic rates because colony activity correlates with its collective metabolism in other ant species (Waters and Harrison 2012). I hypothesize that collective metabolic rates correlate with colony personalities such that more active, aggressive colonies have higher metabolic costs. To test this hypothesis, I plan to extract a subset of workers from colonies with known personality scores and let them acclimate to a custom-built, *Cecropia*-mimicking respirometry chamber in the lab. I will then simulate a vertebrate attack through vibrational disturbance while measuring collective respiration from which I can calculate metabolic rates.

Microbiome and collective behavior – Microbial communities living within and around animals influence important physiological, behavioral, and evolutionary processes (Ezenwa et al. 2012). The functions of microbes are incredibly diverse, ranging from parasitic, to antibacterial, to mediating nutrient transfer. Knowing the identity and abundance of these functional groups and how they differ between colonies will lead to important hypotheses regarding colony behavior and fitness. Working in collaboration with Emily Meineke at Harvard University, I collected culture samples throughout my research. Preliminary analysis from samples suggest bacterial communities differ among nest regions within a colony, as in different rooms of human homes (Dunn et al. 2013). This suggests that colonies may regulate the microbial communities to perform different functions around the nest. Looking forward, I will compare colony microbiomes before and after transplanting them to the greenhouse, which will allow me to *experimentally*

test to what extent individual colonies promote consistent microbial communities in their host trees, and if certain functional groups correlate with certain colony behaviors. The power of this transplant manipulation in an ecological study is unique and lends itself to high impact discoveries.

Genetic basis of aggression – Differences in individual behavior are often driven by genetic differences. Which genes are responsible for colony aggression? The foraging gene (*for*) is a promising candidate because it can influence several behaviors across many taxa, and can lead to differences in colony-level activity (Bockoven et al. 2017). This gives insight into the genetic mechanisms driving personality differences. I plan to investigate the relationship between foraging gene expression and colony aggression in *Azteca-Cecropia* mutualism. Furthermore, simply feeding ants a diet that contains cGMP, the activation molecule for the foraging pathway, can increase colony-level aggression (Malé et al. 2017). I plan to test whether the *Cecropia* hostplant food bodies contain a functional analog to cGMP that can manipulate colony behavior, leading to increased plant protection. In collaboration with Bill Wcislo and the Global Ant Genomics Alliance, I plan to sequence the genome of *A. constructor*, which is the first step in this project, and will open many other lines of research focusing on the genetic basis of colony behavior.

Behavioral variation and community dynamics – Despite the breadth of research on consistent individual differences, few studies focus on the impact of individual variation across populations, species, and communities (Modlmeier et al. 2015). Around the Panama Canal Area, there are at least three species of *Azteca* competing to inhabit four species of *Cecropia* that exist sympatrically. Do species and colony behavior interact

and lead to competitive advantage? Thus far, I have shown that collective personality exists in *A. constructor*. Using similar methods, I plan to determine the degree of behavioral variation, consistency, and personality across colonies of *A. alfari* and *A. isthmica*. I will track colony density, species abundance, and host plant associations and preferences. Studying collective personality in this context will give insight about the evolution and maintenance of consistent behavioral differences, community dynamics, and the balance of sympatric multispecies assemblages.

Pantropical comparisons across symbioses – How widespread is colony personality across ant-plant symbioses and what role does it play in mutualism dynamics of other convergent systems? I plan to study colony behavior in the mutualisms of other *Azteca-Cecropia* in Peru, *Pseudomyrmex*-Acacia in Panama, and *Crematogaster-Macaranga* in Borneo. In 2016, I scouted out locations and contacts in Manu, Peru to investigate the complex *Azteca-Cecropia* community there – at least seven or eight *Cecropia* exist sympatrically. The large elevational gradient from lowland Amazonia to Andean forest provides a perfect template to study how climate may influence personality structure. I will study two *Pseudomyrmex* species living in Acacia trees that are common and abundant around the Canal Zone of Panama. I am planning a scouting expedition to Borneo in July of 2018 to develop locations and contacts for studying the remarkably convergent *Crematogaster-Macaranga* mutualism found there.

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APPENDIX A
WHAT ABOUT SLOTHS?





The best way to see a sloth in the tropics is to scan large Cecropia trees, looking for a still, amorphous lump. The common knowledge is that they spend most of their time in Cecropia trees, preferring their leaves. But do they? It may be just a simple observation bias given that the minimal, elegant structure of the tree provides the best chance to spot something that doesn't fit. Either way, what I'm really interested in is their interaction with Azteca ants. Do ants somehow ignore these slow-moving beasts? Or do the sloths ignore the potential onslaught? Or perhaps colonies differ in their response to sloths. I've gathered minimal anecdotal evidence for any of these ideas because it's hard to get up close to observe what's happening. I have seen sloths get deterred from a tree before they even reach the leaves, and I've seen sloths eating at their leisure. Perhaps it is the sloths that vary in boldness or pain tolerance. Experiment planning is underway.

APPENDIX B

HOWLER MONKEYS ATTACK



These howler monkeys have very different foraging approaches depending on which Cecropia tree they are feeding on. In the first shot, the monkey seems to be minimizing its contact with the plant by reaching out from another tree, retreats quickly after snapping off the soft meristem, and presumably scratches off the attacking Azteca ants. In the second shot, the monkey is chomping at its leisure, lounging on the Cecropia tree itself. Perhaps these monkeys are being met with differing levels of aggression by the resident Azteca guardians . . .

Video: <https://youtu.be/3DvA5fnAvXE>

APPENDIX C
COAUTHOR APPROVAL

All coauthors of published chapters granted permission to use those chapters in this dissertation.

APPENDIX D

SUPPLEMENTARY MATERIALS FOR CHAPTER 2

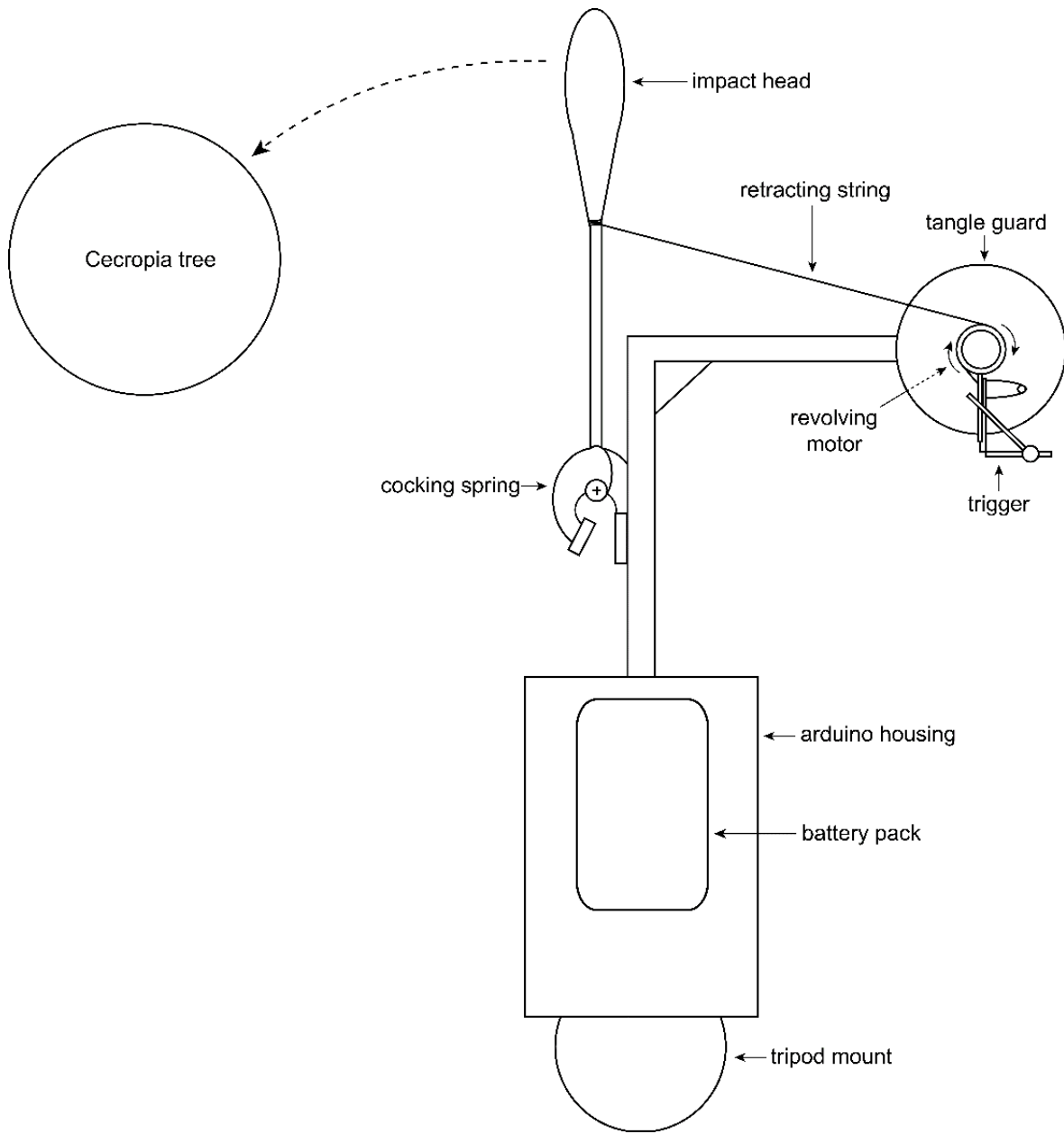


Figure B.1. A diagram of the Flick-O-Matic 2.0, designed to deliver flicks of constant force and timing to *Cecropia* trees to simulate a vertebrate attack.

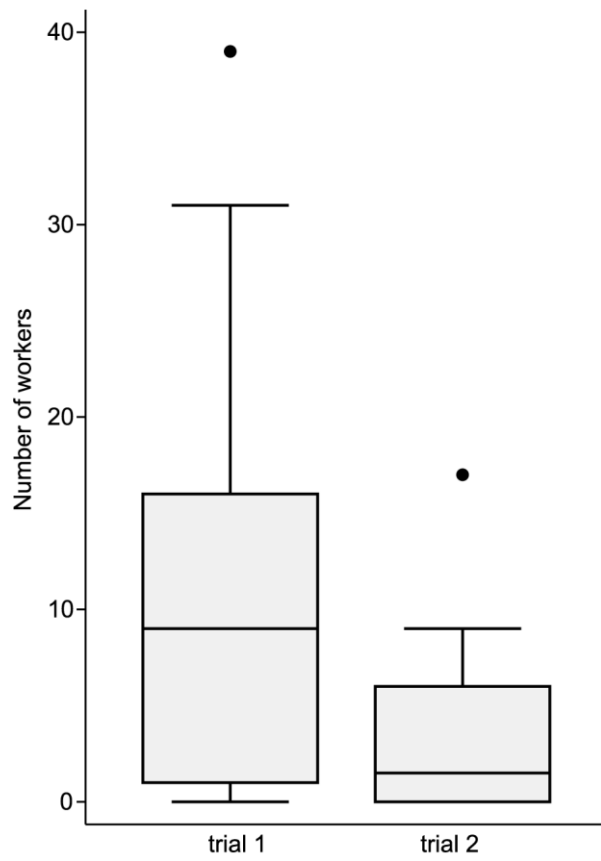


Figure B.2. Colonies tend to have more workers explore during the first of two trials (paired t-test, $p < 0.05$).



Video B.1. *Azteca constructor* colonies differ substantially in their response to range of behavioral traits. Here, two similar-size colonies respond to vibrational disturbance from a "flick-o-matic" robot out of the frame below. After 20 seconds, red dots appear in the top left to indicate when the flicking begins. Shown here is a video still. Full video can be viewed at this manuscript's supplementary materials page or youtube:

<https://www.youtube.com/watch?v=hSR4rWSKTCQ>.

Table B.1. A summary of the principal component analyses for consistent behavioral traits.

	PC1	PC2
Eigenvalue	1.934	1.065
Variance Explained	48.3%	26.6%
Loading Scores		
Patrolling	0.620	0.236
Vibrational disturbance	0.351	0.731
Intruder Response	0.511	0.262
Leaf Damage Response	0.482	-0.610

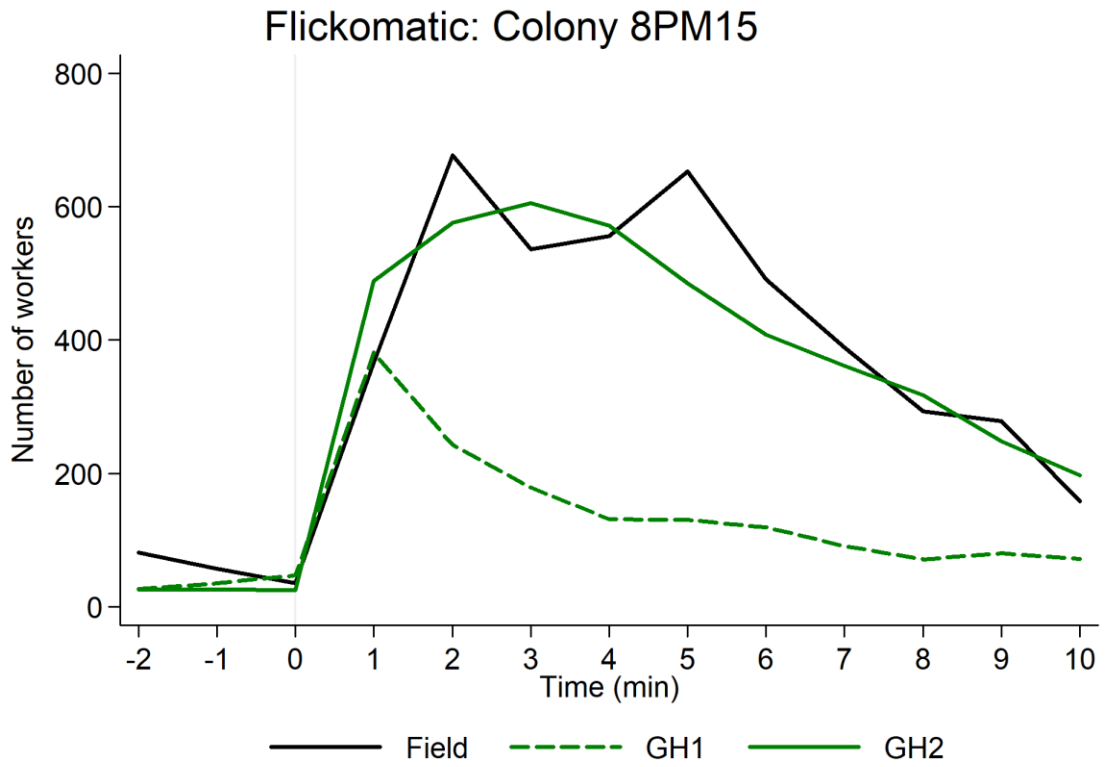
Table B.2. A summary of the principal component analyses for consistent behavioral traits excluding the colony that did not discover the leaf damage.

	PC1	PC2
Eigenvalue	1.937	1.071
Variance Explained	48.4%	26.8%
Loading Scores		
Patrolling	0.619	0.299
Vibrational disturbance	0.356	0.726
Intruder Response	0.492	0.294
Leaf Damage Response	0.498	-0.598

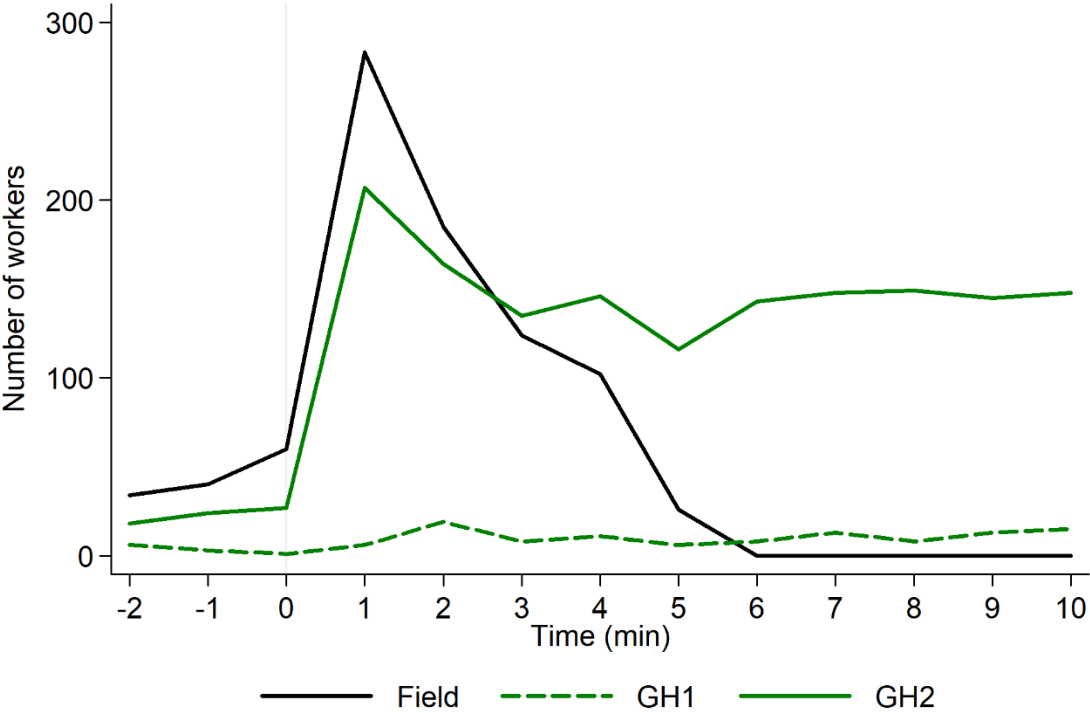
APPENDIX E

BEHAVIORAL CONSISTENCY ACROSS COLONY TRANSPLANTS

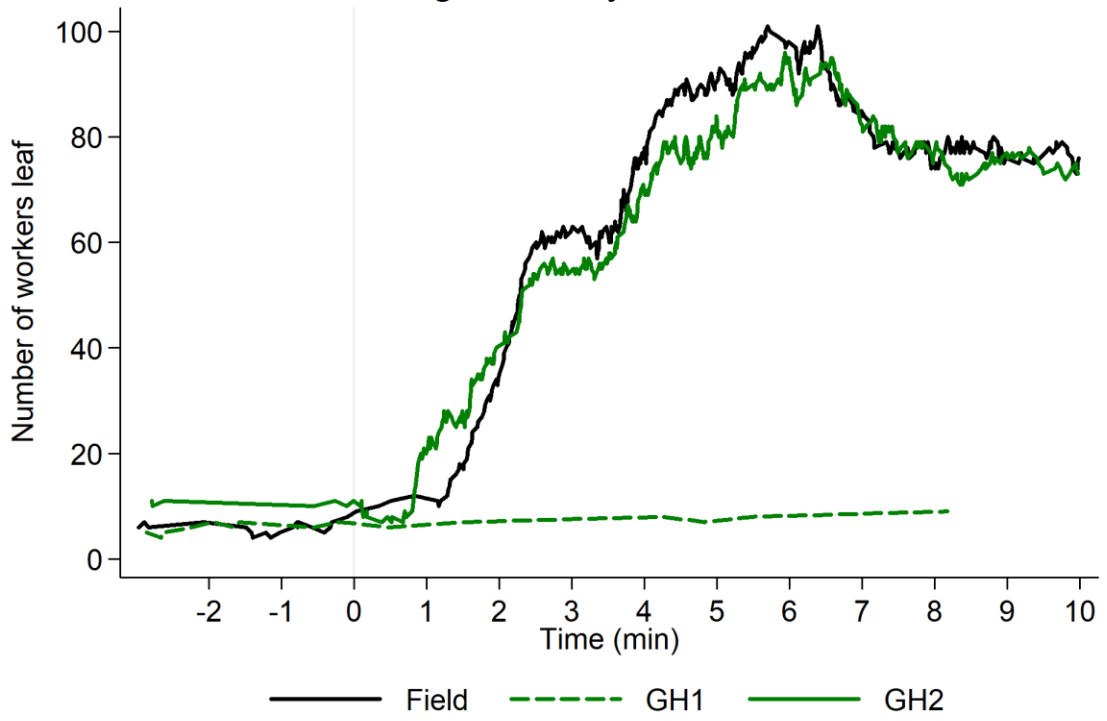
Examples of colonies that behaved *consistently* for different traits from the field assessment to the second greenhouse assessment.

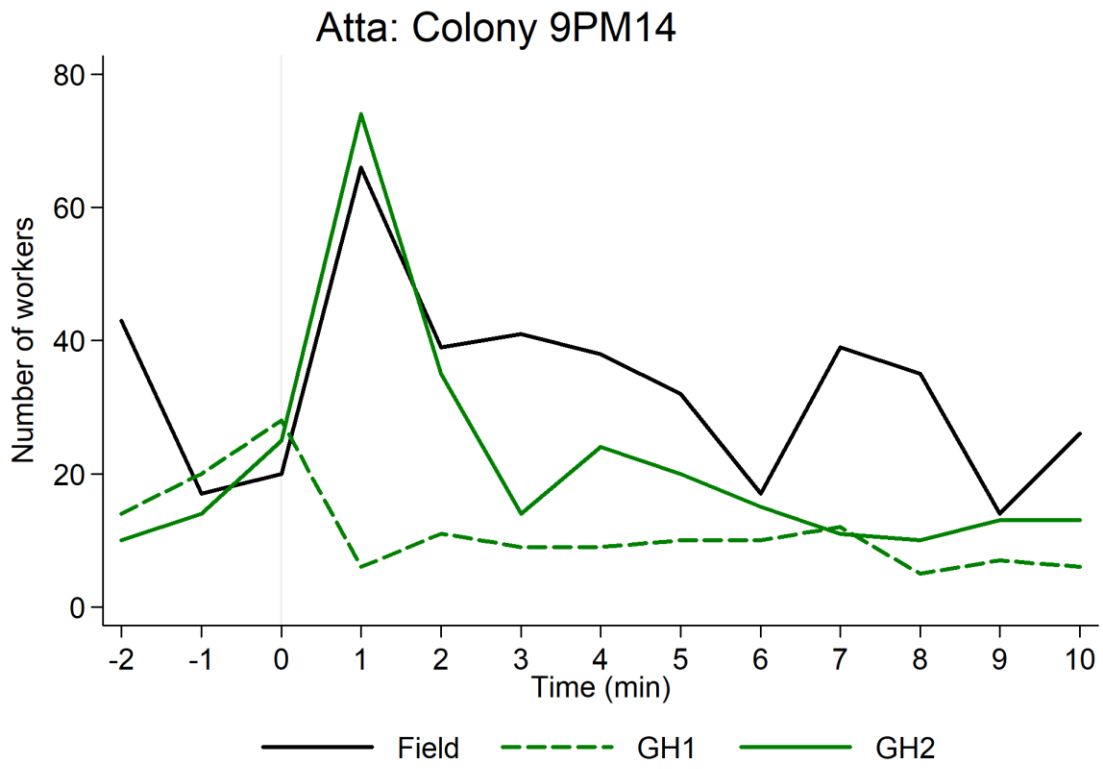


Army Ant: Colony 34PM15

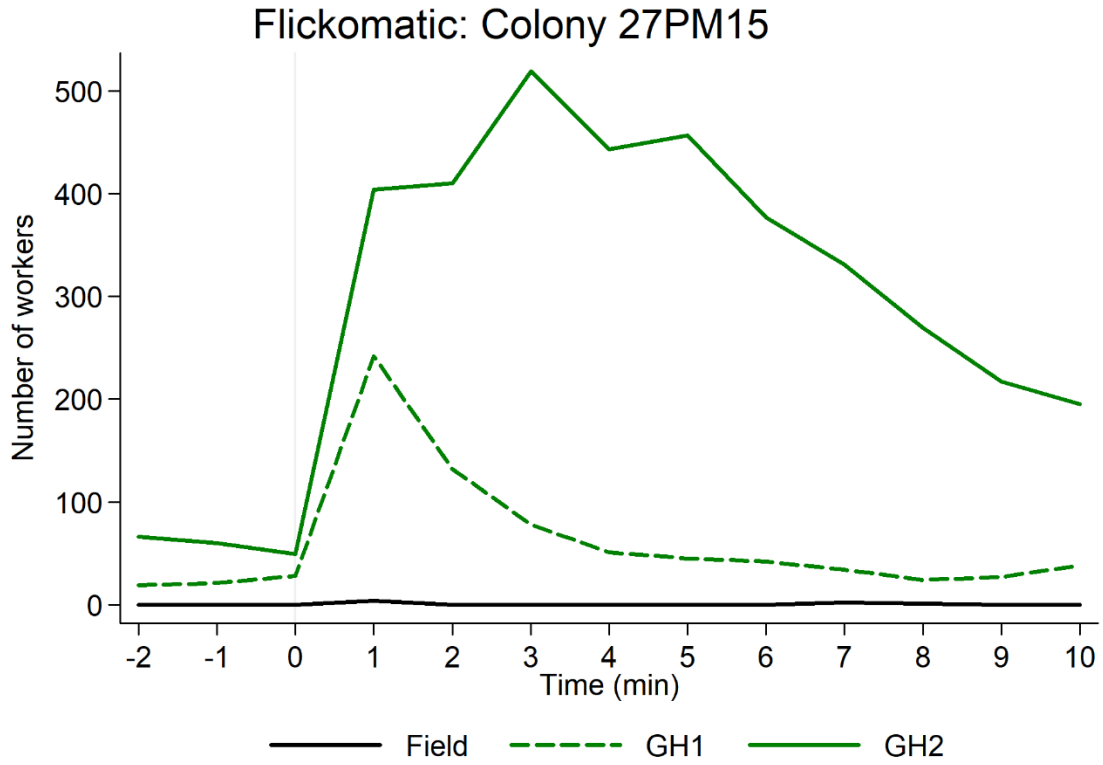


Leaf Damage: Colony 7PM15

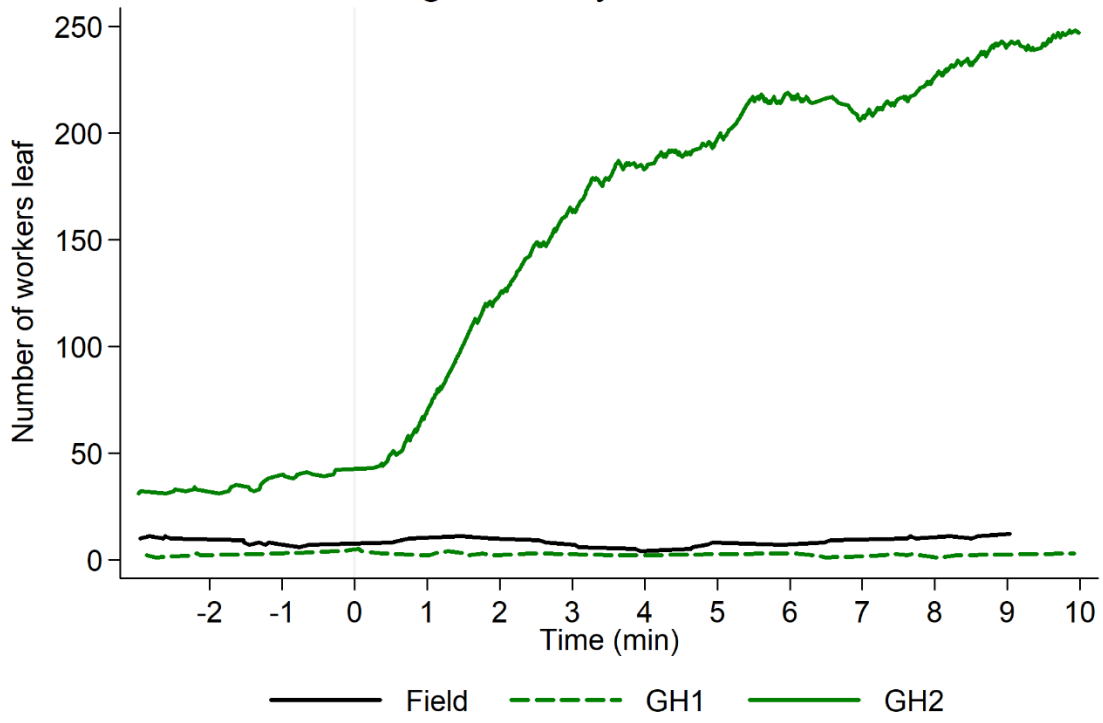


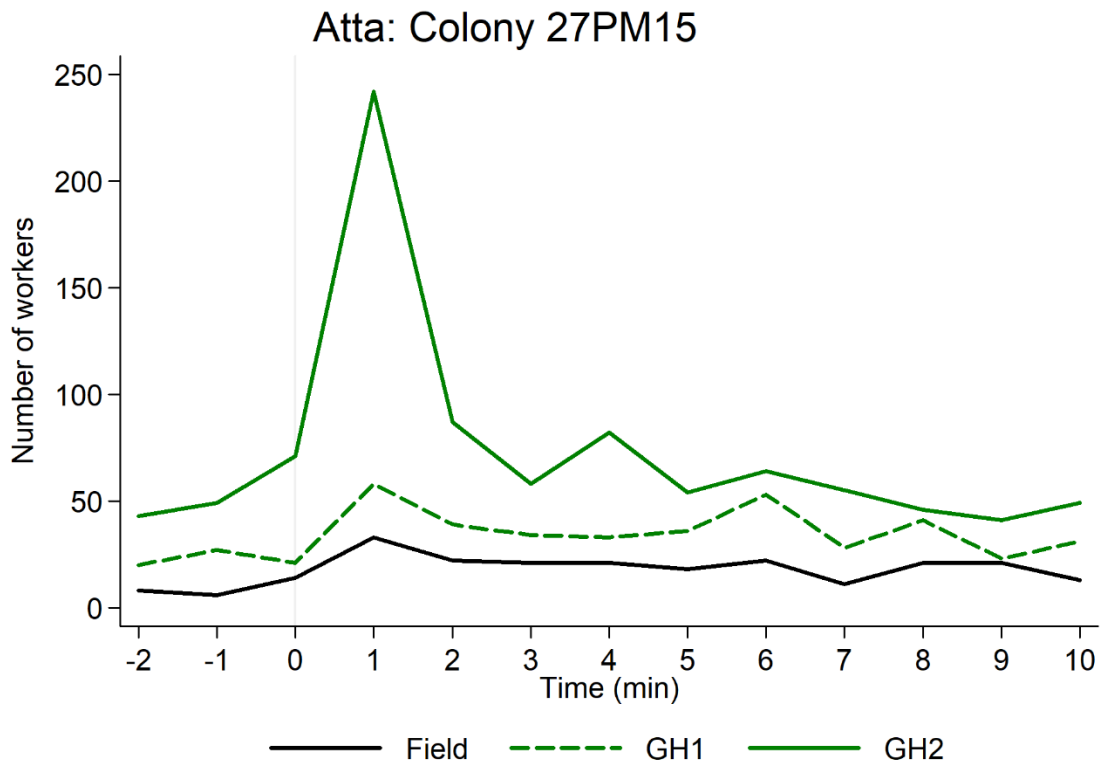


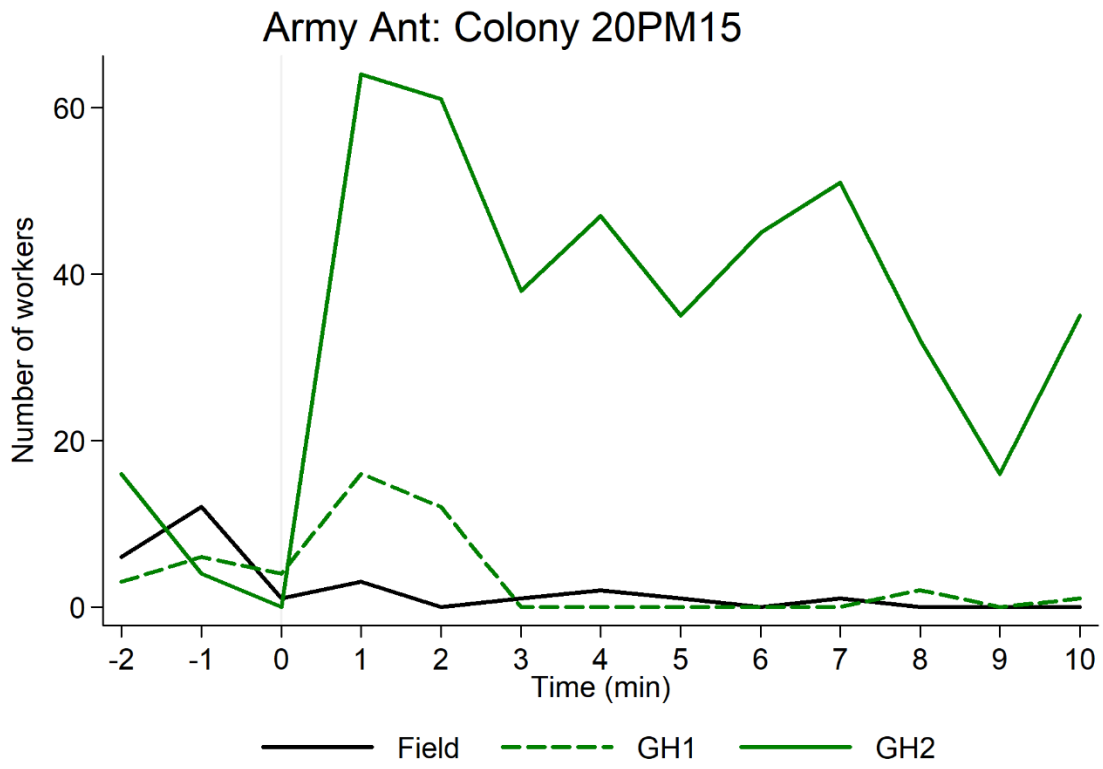
Examples of colonies that behaved *inconsistently* for different traits; responses increased in the greenhouse.



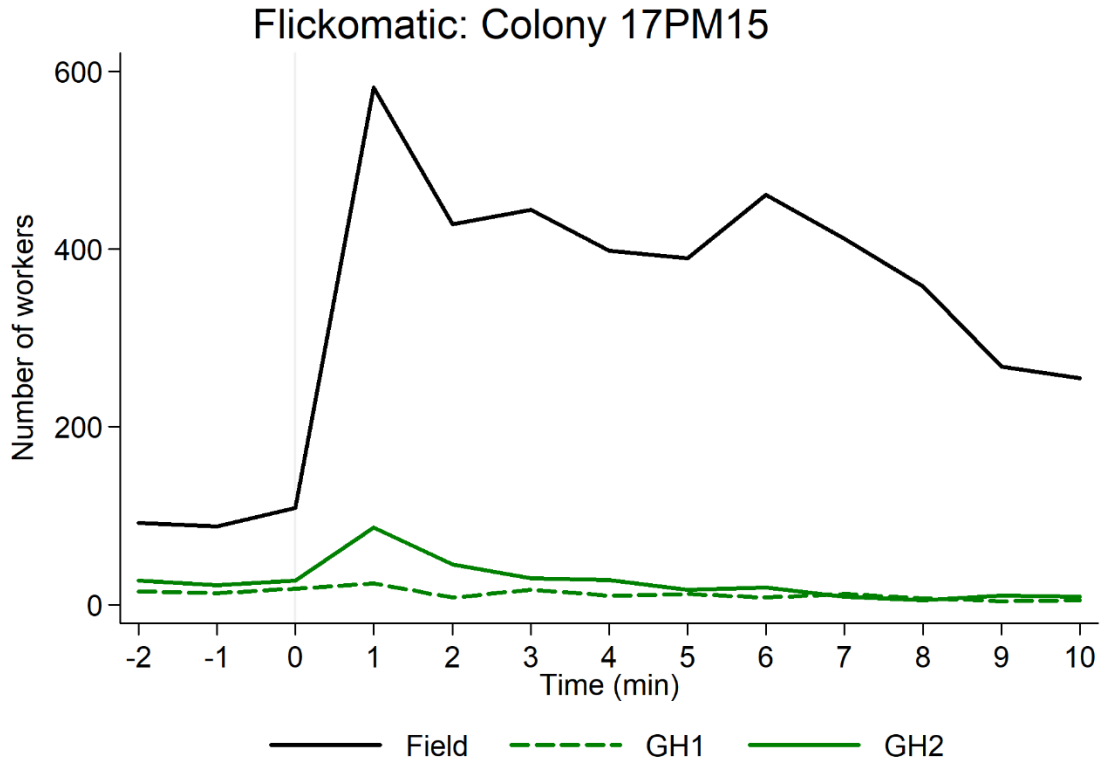
Leaf Damage: Colony 12PM14

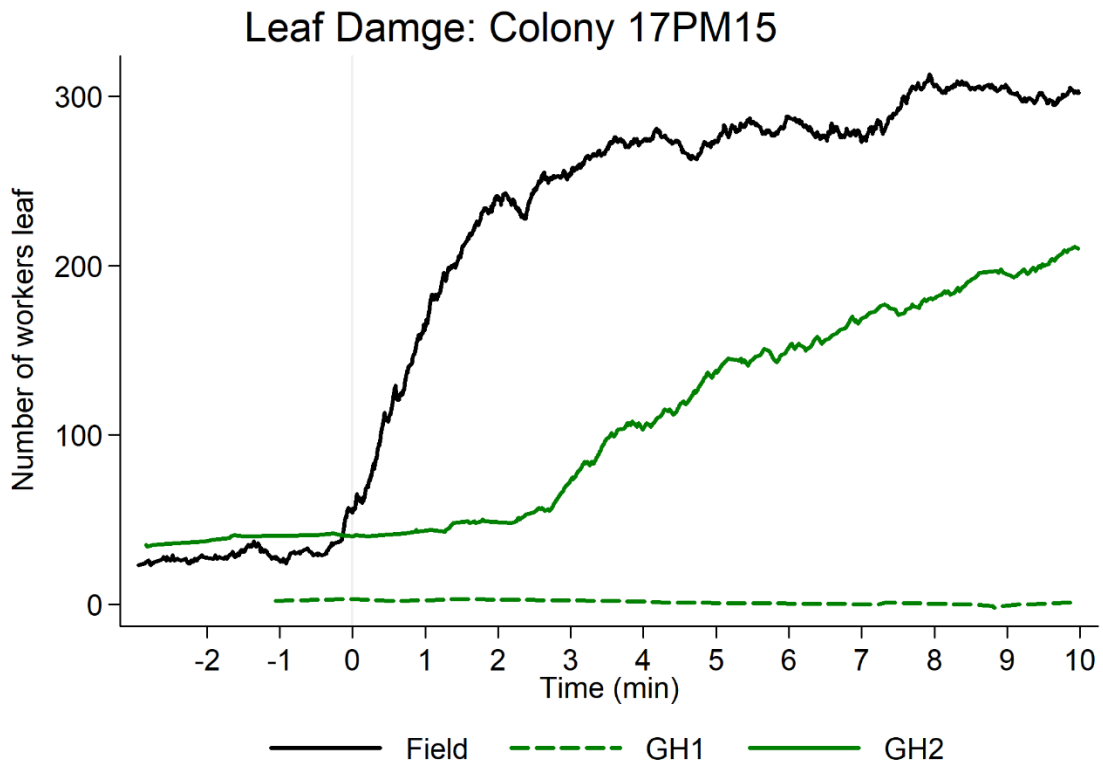


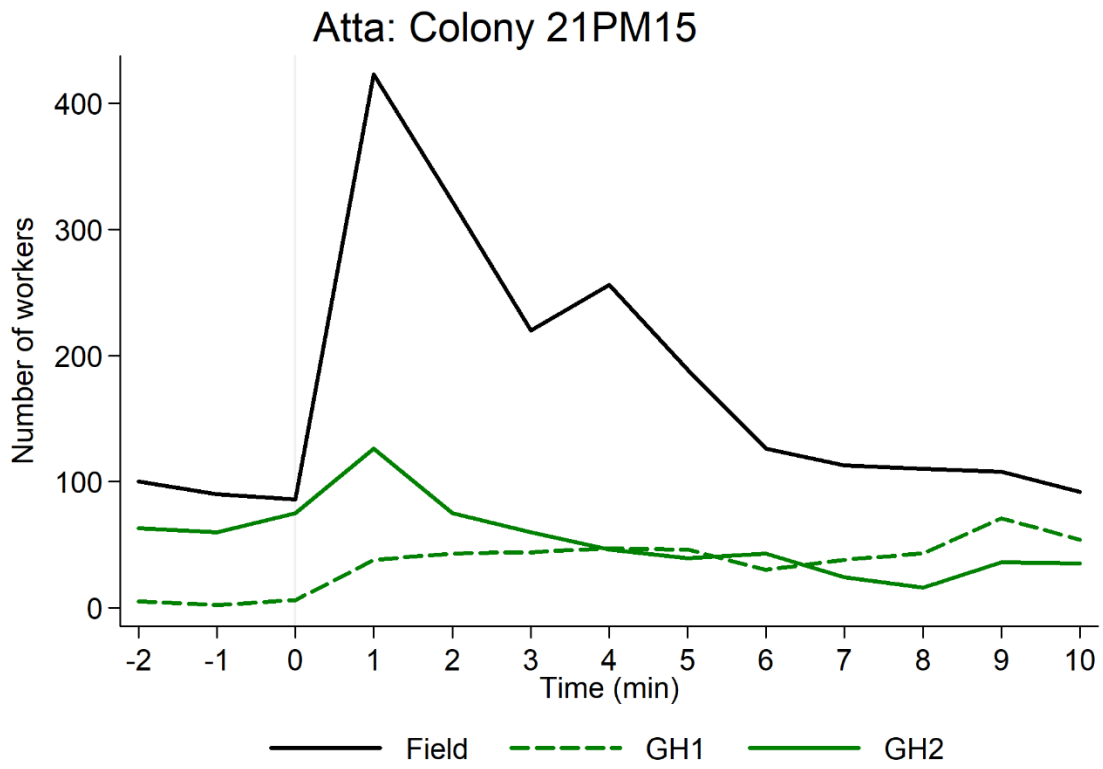




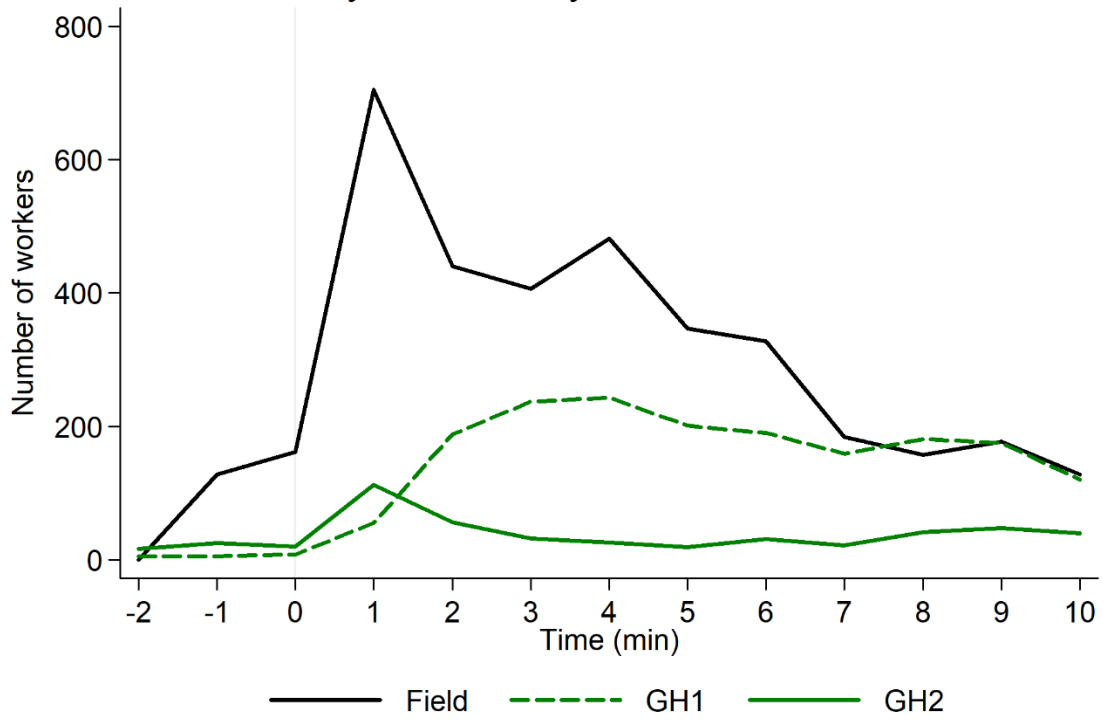
Examples of colonies that behaved *inconsistently* for different traits; responses decreased in the greenhouse.







Army Ant: Colony 17PM15



APPENDIX F

ANTEATERS: A NEGLECTED COST TO ANT-PLANTS?



The benefits of hosting an ant colony often outweigh the costs for *Cecropia* trees, which is why the mutualism exists in the first place. Typically, we think of costs in terms of the services the plant provides for the ants; producing food bodies and allowing sap-sucking scale insects to feed. In one extreme example, some species of ants trim the flowers of their plants, reducing fitness in their *Cordia* hosts.



However, a possible cost that has received less attention is anteater attraction. Hosting a colony invites the risk of a potentially catastrophic anteater attack, especially for younger, developing *Cecropia* trees. I have gathered a few anecdotal observations over the years where I arrive at a long-monitored *Cecropia* tree, ready to start a behavioral experiment, and find a dismal scene: the tree ripped apart, the stem snapped in half, internodes exposed, ants scattered, and leaves uneaten. I have yet to catch a culprit red-handed, but it was clear the beast was interested in the ants, not the plant. Furthermore, the incisions that opened the internodes looked like the work of precise claws and not brute force, pointing to the tamandua, an arboreal anteater. Regardless of the perpetrator's identity, the plants clearly suffered a serious blow merely by being between the predator and its prey. A setback like this could be detrimental for a pioneer plant in the fast-paced, competitive environment of a light gap.



Cecropia trees might be a particularly susceptible ant-plant because Azteca ants don't have venomous stings like *Pseudomyrmex* ants in Acacia trees. Though Azteca are fast and bitey and swift to remove insects, they are likely less effective at deterring large vertebrates like anteaters, leaving their tree exposed to a big risk-by-association.

Many questions remain - how often does this happen? Can ants and plants recover? Do anteaters target certain sizes or ages of trees? Of course, these ponderings must be tested. Experimental planning is underway.