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To the Graduate Council:

I am submitting herewith a dissertation written by Katharine Lisa Stuble entitled "Ant Community Dynamics and the Effects of Global Warming." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Nathan J. Sanders, Major Professor

We have read this dissertation and recommend its acceptance:

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# Ant Community Dynamics and the Effects of Global Warming

A Dissertation Presented for the  
Doctor of Philosophy  
Degree  
The University of Tennessee, Knoxville

Katharine Lisa Stuble  
May 2013

To Martin – never give up

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## ABSTRACT

This dissertation seeks to provide an understanding of how species coexist and, further, how climate change may alter communities by acting on the mechanisms that promote coexistence. Specifically, I examined coexistence among ant species in eastern deciduous forests and the effects that warming may have on foraging activity. Through a series of field observations, I sought evidence for the importance of four of the most commonly cited mechanisms for coexistence among ant species: the dominance – discovery tradeoff, the dominance – thermal tolerance tradeoff, spatial segregation, and niche partitioning. In this system, I did not find evidence for any of these mechanisms, but did find evidence that ant species were segregating the time of day during which they forage. Through an experimental temperature manipulation, I examined the potential effects of climatic warming on ant foraging behavior. I found warming to alter overall rates of foraging, as well as species-specific rates. The relative effects of temperature on foraging rates were predictable based on the thermal tolerance of the species. Finally, I examined the potential for these altered levels of foraging to cause shifts in rates of ant-mediated seed dispersal, providing an indirect mechanisms via which climatic warming may alter the plant community. Despite the observed shifts in ant activity, however, I did not find rates of seed dispersal to vary across temperature treatments. In sum, this dissertation suggests that the mechanisms promoting coexistence among ant species are complex and likely differ from place to place, but that segregation of foraging times may be important in some cases. Additionally, climate change is likely to affect ant communities by altering foraging behavior variably across species, but this may not have direct consequences for the plant community as a result of shifts in rates of seed dispersal.

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## INTRODUCTION

Ecologists have long sought to understand why species occur where they do and how species coexist in local communities (MacArthur 1958, Hutchinson 1959). As the global environment has begun to rapidly change in the past century, a new question has arisen: how might climate change alter species distributions and the ability of species to coexist (Walther et al. 2002, Lavergne et al. 2010, Walther 2010, Harley 2011)? My research seeks to understand both the basic mechanisms underlying the maintenance of diversity within communities, as well as how global change may alter these communities and the services they provide. I use both natural and manipulative experiments with ants to examine how species coexist and how global change drivers alter community dynamics. Specifically, I use ant communities in eastern deciduous forests to 1) examine the importance of a suite of commonly cited mechanisms in mediating coexistence among ant species, 2) understand the potential for climatic warming to alter rates of foraging activity among ants, and 3) examine the effects of climatic warming on rates of ant-mediated seed dispersal.

Understanding community assembly under current conditions allows us to better predict how contemporary global change phenomena may disassemble these same systems. Ant communities are commonly considered to be structured by competition for food (Parr et al. 2005, Parr and Gibb 2010), but the exact mechanisms that allow multiple species to co-occur are unclear. Several specific mechanisms have been suggested, including evolutionary tradeoffs and niche segregation, and my research seeks to clarify the relative importance of these.

In chapter 2, I examine the relevance of a suite of ant coexistence mechanisms including the dominance-discovery tradeoff, the dominance-thermal tolerance tradeoff, spatial segregation, temperature-based niche segregation, and temporal niche segregation in an eastern deciduous ant community. I found no evidence for the first four coexistence mechanisms in the study system. However, I did find evidence that species were segregating time-of-day. Specifically, behaviorally dominant species foraged primarily during the night, while subordinate species were much more likely to be found on food baits during the day.

Global climate change poses a substantial threat to organisms and ecosystems worldwide (Stachowicz et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Malcolm et al. 2006, Wallisdevries and Van Swaay 2006, Brander 2007). However, less is known regarding the mechanisms driving these changes, including the importance of biotic interactions (Davis et al. 1998, Araújo and Rahbek 2006) as well the consequences of these shifts on ecosystem processes. Thus, experimental tests are useful in examining the impacts of climate change. My dissertation research involves experimental manipulation of air temperature in two deciduous forests in the eastern US to examine the impacts of warming on ant assemblages and the seed dispersal services they provide.

In chapter 3, I use an experimental temperature manipulation to examine the impacts of warming on patterns of ant foraging activity at two geographic locations. Results from this study suggest that the effects of warming will be both site and species-specific. The more southerly site exhibited an increase in overall ant foraging activity with increasing temperature while the northern site showed no relationship between foraging activity and temperature treatment. Examining the effects of warming on individual species, I found species to respond in

accordance with their thermal limits. Specifically, species with higher critical thermal maxima foraged more heavily under warmer conditions than did species with lower critical thermal maxima. These findings may aid scientists in predicting the responses of organisms to climatic warming.

In chapter 4, I examined the potential for climatic warming to alter seed dispersal in eastern deciduous forests. I examined both overall and species-specific rates of seed removal with the expectation that the above-observed changes in rates of ant foraging activity would result in altered numbers of seeds removed by ants under experimentally warmed conditions. However, I found no changes in rates of seed removal (either overall or by individual species) as a result of experimental warming. This result suggests that climatic warming may have a lesser indirect impact on the plant community via altered rates of ant-mediated seed removal than might be expected based on the impacts of warming on ants alone.

Overall, my dissertation examines the questions regarding the structure and function of ant communities, both under ambient conditions as well as under experimental warming. I suggest that much still remains to be learned regarding the factors regulating the coexistence of species. Prominent methods of coexistence may vary across ecosystems and examinations of multiple methods of coexistence within a single system can be valuable. Additionally, climatic warming has the potential to alter ecological processes. However, these effects can be difficult to predict without the use of experimental manipulations.

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# **CHAPTER I**

## **TRADEOFFS, COMPETITION, AND COEXISTENCE IN EASTERN DECIDUOUS FOREST ANT COMMUNITIES**

### **Abstract**

Ecologists have long sought to explain the coexistence of multiple potentially competing species in local assemblages. This is especially challenging in species-rich assemblages in which interspecific competition is intense, as it often is in ant assemblages. As a result, a suite of mechanisms has been proposed to explain coexistence among potentially competing ant species: the dominance – discovery tradeoff, the dominance – thermal tolerance tradeoff, spatial segregation, temperature-based niche partitioning, and temporal niche partitioning. Through a series of observations and experiments, we examined a deciduous forest ant assemblage in eastern North America for the signature of each of these coexistence mechanisms. We failed to detect evidence for any of the commonly suggested mechanisms of coexistence, with one notable exception: ant species appear to temporally partition foraging times such that behaviorally dominant species foraged more intensely at night, while foraging by subdominant species peaked during the day. Our work, though focused on a single assemblage, indicates that many of the commonly cited mechanisms of coexistence may not be general to all ant assemblages. However, temporal segregation may play a role in promoting coexistence among ant species in at least some ecosystems, as it does in many other organisms.

## Introduction

A fundamental and long-standing goal in ecology has been to determine how seemingly similar species coexist in local communities (MacArthur 1958; Hutchinson 1959). Despite ever increasing attention on neutral processes (Hubbell 2001; Rosindell et al. 2011), evidence for the importance of three general mechanisms of coexistence drawn from niche theory continues to accumulate in the literature: environmental partitioning (Schoener 1974; Wright 2002; Levine and HilleRisLambers 2009), tradeoffs (Wright 2002; Kneitel and Chase 2004), and spatial segregation (Kunz 1973; Shigesada et al. 1979). At least since 1958 (MacArthur 1958), ecologists have sought to document how partitioning of environmental niches in communities promotes coexistence (Schoener 1974; Dueser and Shugart 1979; Chase and Liebold 2003; Silvertown 2004). Similarly, tradeoffs (i.e., inverse relationships between functional traits among organisms) might also promote segregation among species (Tilman 1994; McPeck et al. 2001; Levine and Rees 2002; Yurewicz 2004) if conditions are such that no one species is dominant under all conditions. Finally, species may be segregated in such a way that promotes coexistence, either at small or large spatial scales (Gotelli and McCabe 2002, Gotelli et al. 2010). While each of these mechanisms for coexistence may occur in at least some systems, their relative importance within a single community has largely been unexamined.

In ants, many species often coexist in local communities. For example, Silva and Brandão (2010) reported 30 species in a square meter of leaf litter in eastern Brazil, and Andersen (1983) documented more than 80 species in 0.25 ha in semi-arid northwestern Victoria, Australia. In temperate systems, 10 species can occur in a square meter of litter, and 22 species might coexist in a 0.25 ha plot (Sanders et al. 2007c). Superficially, such diversity is surprising since

competitive exclusion is often cited as playing a major role in controlling species density in ants, which often compete for food resources (Parr et al. 2005; Parr and Gibb 2010). Considerable research has focused on the coexistence of ant species and the factors that may be involved in promoting coexistence in ant communities (Parr and Gibb 2010).

To our knowledge, no study has simultaneously examined a suite of tradeoffs and the potential for niche partitioning in a single ant community (or a single community of any taxon for that matter). In this study, we examine evidence for a suite of mechanisms with the potential to facilitate coexistence within a deciduous forest ant community in the southeastern United States. These mechanisms, described below, include the dominance – discovery tradeoff, the dominance – thermal tolerance tradeoff, spatial partitioning, temperature-based niche partitioning, and temporal niche partitioning.

### **Dominance – discovery tradeoff**

Tradeoffs in different ecological functions or tolerances among species are one of the most common explanations for coexistence in communities (Kneitel and Chase 2004). These tradeoffs may be universal, bounding similar taxa to the same tradeoffs despite being spatially isolated (Tilman 2011). The dominance-discovery tradeoff suggests that coexistence is promoted by a tradeoff in behavioural dominance and resource discovery ability among ant species (Fellers 1987; Davidson 1998; Adler et al. 2007). Behaviourally dominant ant species (typically defined as those species that can readily defend resources from other ant species) may find food more slowly than do subordinate species (Fellers 1987). A dominance – discovery tradeoff can promote coexistence if the species able to find resources more quickly tend to be poor at defending those resources against other ant species while species that find resources slowly tend

to be more aggressive and are better able to dominate a food resource. A negative correlation between resource discovery ability and some measure of dominance among a suite of coexisting ant species is the signature of this tradeoff. A recent synthetic review of the subject suggests that there is only limited support for this tradeoff (Parr and Gibb 2011).

### **Dominance – thermal tolerance tradeoff**

The dominance – thermal tolerance tradeoff posits that behaviourally dominant ant species can forage under only a relatively narrow window of temperatures, while behaviourally subordinate ants can, and indeed need do, forage over a wider range of temperatures in order to avoid interference by dominant species (Cerdá et al. 1998). The signature of this tradeoff is a negative correlation between the thermal tolerance of a species and an estimate of its behavioural dominance. Evidence for the dominance – thermal tolerance tradeoff in ant assemblages comes from a variety of ecosystems (Cerdá et al. 1997; Cerdá et al. 1998; Bestelmeyer 2000; Lessard et al. 2009).

### **Spatial partitioning**

As in many other species (Gotelli and McCabe 2001) ants may segregate space, thereby creating spatial mosaics, or checkerboard distributions of species within sites. Such non-random patterns of spatial distribution may indicate strong competition (Majer et al. 1994; Albrecht and Gotelli 2001), though it is also possible that such patterns simply reflect differences among species in habitat/microhabitat preference (Ribas and Schoereder 2002). Spatial segregation can promote coexistence by reducing the frequency of interspecific encounters among species. The spatial arrangement of colonies appears to be structured by competition in several systems (Levings and Traniello 1981; Ryti and Case 1992) and may influence variation

in the use of patchy resources such as those mimicked by baits (Albrecht and Gotelli 2001). Spatial segregation can be particularly pronounced among behaviorally dominant ant species and has been found, for example, to produce a mosaic pattern in the distribution of dominant arboreal ants (Majer et al. 1994; Pfeiffer et al. 2008). Evidence for spatial partitioning typically includes comparison of observed co-occurrence to the null expectation of co-occurrence in the absence of the proposed mechanism (Gotelli and Graves 1996). Observed overlap among species that is lower than expected is considered evidence that species are spatially segregated. However, several studies have failed to find evidence for spatial partitioning at local scales (Gotelli and Ellison 2002; Ribas and Schoereder 2002; Blüthgen and Stork 2007; Sanders et al. 2007a).

### **Partitioning of environmental niches**

In addition to segregating spatially, ants and other species may segregate among various aspects of microhabitat or microclimate, including temperature (Torres 1984, Kaspari 1993). For instance, species in some systems segregate among times of day at which they forage (Kronfeld-Schor and Dayan 1999; Albrecht and Gotelli 2001; Gutman and Dayan 2005). Several studies have found strong temporal segregation of ant species, and, for the most part cite temperature as the factor regulating these patterns (Fellers 1989; Cerdá et al. 1997; Albrecht and Gotelli 2001). Temperatures themselves, independent of time of day, can also be segregated by ant species (Torres 1984; Retana and Cerdá 2000).

The importance of each of these potential coexistence mechanisms has been examined alone, but never, to our knowledge, have they all been tested simultaneously in any single system, whether of animals, plants or other taxa. Here, we use observational data to search for signatures of each of these proposed mechanisms within a single ant assemblage in an eastern

deciduous forest to determine which, if any, may play a role in promoting coexistence among twelve common ground-foraging ant species.

## **Methods**

### **Study site**

We conducted this study within the Eno River Unit of Duke Forest, North Carolina, USA (35° 52' N, 79° 59' W, 130 m elevation). The area consists of an 80-year old oak-hickory forest. The mean annual temperature is 15.5°C, and the area receives approximately 1140 mm of precipitation annually.

### **Ant Baiting**

We sampled the ant community in the forest at 24 randomly chosen plots, spaced at least 50 m apart, within Duke Forest. The 50-m spacing is enough to assure that ants likely do not interact among plots. Within each plot, we arranged 12 baits (approximately 50 mg of cat food [14% protein, 3% fat; 1.5% fiber] on a laminated white index card) directly on the leaf litter in a 3 × 4 grid, spaced 10 m from one another. Baits such as the ones we used here are commonly used in studies of ant community ecology to simulate naturally occurring, patchy, protein-rich food resources that ants frequently compete for (Fellers 1987; Andersen 1992; Cerdá et al. 1997; Albrecht and Gotelli 2001; Parr et al. 2005; Feener et al. 2008; Lessard et al. 2009).

We sampled four of the plots once an hour for 24 hours during non-rainy weather in June and July of 2009, always starting trials at 0800 hours. We sampled the other 20 plots every 15 minutes for 3 hours from May through July of 2009 between the hours of 0830 and 2000.

Sampling during this time period assured that our sampling would overlap the seasonal period of

peak ant foraging (Dunn et al. 2007). Only one plot was sampled per day and each plot was sampled only once during the course of the study. During each observation, we counted the number of workers of each ant species present on the bait, noted the first interspecific interaction and indicated whether it was aggressive or neutral. Aggressive interactions were those in which one ant was expelled from the bait. For the aggressive interactions we noted which species was dominant and which was subordinate. We classified a species as being behaviorally dominant (or winning) if after interacting with the other species it remained on the bait. The subordinate species was the species that left the bait following the interspecific encounter. Interactions in which neither ant left the bait following the interaction were recorded as neutral. At each bait, we also recorded the ground surface temperature of the leaf litter just outside of the four corners of the index card using a handheld infrared thermometer (Raytek® Raynger ST). These four surface temperatures were then averaged to estimate the surface temperature of the ground in the vicinity of the bait at the time of the observation. We observed baits at night using a red light that was shone on the bait for fewer than 30 seconds at each check.

### **Discovery Ability**

To determine the relative ability of each species to discover food resources, we positioned six baits in a circle (a discovery array) with a diameter of approximately 60 cm and baits spaced 30 cm from one another. Bait cards were not considered independent; rather each array served as an experimental unit. We observed baits continuously and each time an ant discovered a bait, we recorded the time to discovery (the amount of time between the bait being placed and its discovery) and the identity of the species that discovered the bait. The bait and the discovering ant were removed from the circle for the rest of the trial to prevent recruitment to the bait, which

may have influenced discovery of the bait by subsequent species. Trials were conducted for 60 minutes or until all six baits had been discovered and were conducted during both the day and the night. A total of 98 discovery trials were conducted. All trials took place outside of the above-described baiting plots.

In order to account for the influence of relative abundance on discovery ability (described below), we placed a single pitfall trap (55mm diameter by 75mm deep) in the centre of the discovery array 24 hours after the discovery trial was conducted. The pitfall trap was open for 48 hours before we collected and identified all of the ants in the trap. While pitfall traps may be slightly biased toward species that fall into traps more readily than others, pitfall traps do document the relative abundances of the species actively foraging on the surface of the ground (Gotelli et al. 2011), which is the case for the species examined here.

## **Analyses**

For all analyses below, we considered only species that were observed on twelve or more occasions; attempting to estimate foraging conditions for rare species or species that were infrequently observed might have led us to make spurious conclusions. Except where noted, all analyses were conducted using SAS version 9.2.

## **Behavioural dominance rankings**

We created a dominance hierarchy for the ants in the study system using two common methods: (1) the Colley dominance matrix and (2) proportion of aggressive interactions won. The Colley matrix was originally developed for the purpose of ranking American college football teams (Colley 2002) and was first applied to ant communities by LeBrun and Feener (2007). This matrix estimates dominance based on wins and losses as well as the relative strength



of the opponents. A major advantage of Colley matrix relative to methods of ranking species by dominance is that it does not require each species to have interacted with all other species in order to rank them relative to one another. For details on how the Colley matrix is calculated, see Colley (2002). We also calculated a dominance index for each species based on the proportion of aggressive encounters won by that species in the 24 and 3-hour bait observations, combined (Fellers 1987). We found these two methods produced quantitatively similar hierarchies ( $r^2 = 0.90$ ,  $p < 0.01$ ) (Supplemental Table I.7), and so we use the index based on proportion of aggressive encounters won (the Fellers method) as the metric of dominance in all analyses that follow. Additionally, we calculated Bayesian confidence intervals for the dominance index based on the Fellers method for each species using the `binom.bayes` function in the `binom` package in R. As mentioned above, we recorded a species as winning an encounter if it remained on the bait while the losing species left the bait. We included all interspecific interactions with a clear winner and loser in the creation of these dominance rankings. Interactions without a clear winner were not included in the ranking.

### **Discovery ability**

We used two methods to estimate discovery ability. In the first, we determined the total number of baits discovered by a species to provide a colony-level measure of discovery ability. In the second, the number of baits discovered by a species was standardized by the foraging activity of that species (as measured by the number of pitfalls the species fell into) to provide a measure of relative discovery ability. In this second method, a null expectation for the number of baits expected to be discovered by a given species was calculated as:  $(a/b) \times c$ ; where  $a$  is the sum of baits discovered by each species (baits discovered by species 1 + baits discovered by

species 2 + ...);  $b$  is the sum of pitfalls from which each species was detected (species 1 pitfall occurrences + species 2 pitfall occurrences + ...), and  $c$  is the number of pitfalls in which a given species was detected. A line was created by plotting the expected number of baits discovered as a function of the number of pitfall traps in which the species was present, representing the number of baits predicted to be found for a given occurrence of workers. We then calculated the distance from this line to the observed number of baits discovered by each species (the residuals). A positive value for the residual would indicate that the species finds more baits than would be expected based on its abundance while a negative value would indicate that the species finds fewer baits than would be expected based on the number of occurrences. We then used the residual as a metric of discovery ability (LeBrun and Feener 2007).

We also calculated an estimate of discovery time for each species. Using the minimum discovery time for each species at each discovery array, we calculated the median time at which each species discovered baits, along with the 25<sup>th</sup> and 75<sup>th</sup> quantiles. Discovery times were not calculated for species that failed to discover baits.

### **Dominance – discovery tradeoff**

To test the importance of the dominance – discovery tradeoff in this system, we conducted two separate linear regressions. The first of these regressions used the raw number of baits discovered by each species as the measure of discovery ability regressed against dominance while the second used the residuals described above regressed against dominance; this second method corrects for relative abundance of species. A significantly negative relationship would indicate a tradeoff between behavioural dominance and the ability to discover food items by

showing that behaviourally dominant species are less able to discover new food items (Figure I.1).

### **Dominance – thermal tolerance tradeoff**

To assess the importance of the dominance – thermal tolerance tradeoff in this community we first determined the mean, minimum, maximum, and standard deviation of the temperatures at which we observed each species foraging for all observations. We then used a simple linear regression to examine the relationship between each of these factors and the dominance index. A significantly negative relationship between the standard deviation of foraging temperatures and dominance indicates a tradeoff between dominance and the ability to forage at a broad range of temperatures. A positive relationship between minimum temperature and dominance would indicate a tradeoff between behavioural dominance and the ability to cope with low temperatures (Figure I.1).

### **Spatial analysis**

We tested whether species partitioned baits by examining species co-occurrences among individual bait cards during the final hour of observations during the three-hour baiting trials. We used the final hour in order to document the outcome of both discovery and behavioral dominance. A species was considered present if it was detected on the bait at any point during the final hour of the trial. We then used these presence-absence data to calculate a C-score (Appendix I.1) (Stone and Roberts 1990).

### **Temperature-based niche partitioning**

To examine differences in foraging temperature among species we considered foraging activity by each species in each of nine 2°C temperature windows ranging from the coldest

temperature window observed (20 to 22°C) to the warmest window at which foraging was observed (36 to 38°C). For this analysis of niche space, only the 3-hr baiting trails were used in order to eliminate the potentially confounding effects of dramatic shifts in temperature between day and night.

We used the mean percent of maximum number of baits occupied for each species in each temperature class as the measure of foraging activity in a “niche.” We then calculated the Czechanowski index of niche overlap using EcoSim 7.72 (Gotelli and Entsminger 2010) for the community overall, as well as among the five most dominant species, and among the subdominant species. We also classified each species as being either dominant or subdominant and calculated a Czechanowski index between these two groups (Appendix I.2). We categorized the five most dominant species based on proportion wins as dominant and the remaining species as subdominant. We chose to make the split between dominant and subdominant species here as the dominance indices were similarly high among the first five ant species and there was a gap before the remaining species with lower Colley matrices.

### **Temporal niche partitioning**

To examine the potential segregation of foraging times during the day we used data from the 24-hour baiting trials. Each hour was considered an individual unit that could be used by a species, as has been done by other researchers examining temporal niches (Albrecht and Gotelli 2001). Because 24-hour trials started at 0800, the 0800 and 0900 hours were eliminated as they represent the first and second observations after baits were set out and many species were absent from these bait checks. Foraging-time niche analysis was conducted exactly as temperature-

based niche segregation was examined above with the mean percent of maximum number of baits occupied for each species in each hour as the measure of foraging activity in a “niche.”

## Results

We observed a total of 22 ant species in either pitfall traps or on baits in this study, though twelve ant species were common (observed on baits at least twelve times) and subsequently used in the analyses that follow. Additionally, 371 aggressive encounters between species were observed. *Camponotus pennsylvanicus* was the most behaviourally dominant species at baits. However, the 95% confidence intervals around its dominance score overlapped considerably with those of *Crematogaster lineolata*, *Prenolepis imparis*, *Camponotus americanus* and *Camponotus castaneus*, suggesting that these 5 species were generally the dominant species. The least behaviourally dominant ant was *Temnothorax curvispinosus* (Figure I.2, Table I.1). Further, the 95% confidence intervals around the dominance indices overlapped for many species, which is to say many species in this system have similar competitive abilities or, at the very least, more data are needed to discern subtle competitive differences between species.

### Discovery ability

*Aphaenogaster rudis* discovered baits the fastest, with a median discovery time of 1 minute. *Camponotus castaneus* was the slowest, with a median discovery time of 12 minutes when it was the first species to discover the bait (Supplemental Figure I.11). After controlling for foraging activity by calculating the residuals of the relationship between abundance and bait discoveries (a metric of how far a species was from discovering the number of baits that would be expected based on its occurrences in pitfall traps), the species that discovered the highest

number of new baits, given its abundance, was *Aphaenogaster rudis* while *Camponotus pennsylvanicus* discovered the fewest (Table I.1). Two species, *Tapinoma sessile* and *Aphaenogaster lamellidens*, were not observed frequently enough at discovery trials to determine their discovery ability.

### **Dominance-discovery tradeoff**

Contrary to the predictions of the dominance-discovery tradeoff, we found no relationship between dominance and discovery ability among species using either method for determining discovery ability (colony level:  $r^2 = 0.15$ ,  $p = 0.27$ ; worker level:  $r^2 = 0.28$ ,  $p = 0.12$ ).

### **Dominance-thermal tolerance tradeoff**

Ground surface temperatures observed during the day ranged between 19 and 52°C (based, in part, on whether the bait was in direct sunlight or shade), and some ant species foraged across this entire range (Supplemental Figure I.12). However, 90% of instances in which we saw ants at baits occurred between 24 – 28°C, with a mean observed ground surface temperature of 27°C and a median of 26°C. We found no relationship between dominance and mean ( $r^2 < 0.01$ ;  $p = 0.96$ ), minimum ( $r^2 = 0.01$ ;  $p = 0.74$ ), or maximum foraging temperature ( $r^2 = 0.02$ ;  $p = 0.67$ ), nor was behavioural dominance related to the standard deviation of foraging temperature ( $r^2 = 0.01$ ;  $p = 0.83$ ).

### **Spatial analysis**

We used null model analyses to determine whether species were spatially segregated from one another. We found no evidence that ant species were spatially segregated overall, among just the dominant species, or among just the subdominant species (Supplemental Figure

I.13). That is, in all cases, the observed C-score (a measure of co-occurrence) did not differ from the null expectation that species co-occurred randomly with respect to one another ( $p > 0.20$  in all cases).

### **Temperature-based niche partitioning**

Considering temperature (based on 2°C temperature classes) as a possible factor defining the foraging niche, niche overlap was greater than expected by chance for all groups compared: all ants ( $p < 0.01$ ), dominant species only ( $p < 0.01$ ), subdominant species only ( $p < 0.01$ ), and dominants vs. subdominants ( $p < 0.01$ ) (Table I.2).

### **Temporal niche partitioning**

We observed significant niche-differentiation when time of day was examined as the focal niche axis. In the overall ant community, significant niche segregation of foraging time was apparent ( $p = 0.02$ ) (Table I.2). The five most dominant species, however, showed greater niche overlap than the null expectation ( $p < 0.01$ ) (Figure I.3a). Subdominant species also overlapped in foraging times with one another more than would be expected ( $p < 0.01$ ) (Fig. I.3b). Additionally, dominant ants occupied a different niche space than did subdominant ants ( $p < 0.01$ ). Dominant ants in the study system focused the bulk of their foraging effort at night while subdominant ants foraged more during the day (Figure I.4).

## **Discussion**

The idea that interspecific competition drives community assembly and limits local coexistence has been at the core of community ecology (Kneitel and Chase 2004; Tilman 2011). Numerous mechanisms promoting the maintenance of coexistence have been proposed in a wide array of

taxa (MacArthur 1958; Wright 2002; Silvertown 2004). In ants, competitive interactions and a suite of tradeoffs associated with competitive ability and thermal tolerance have long been thought to structure communities (Hölldobler and Wilson 1990; Parr and Gibb 2010). Generally speaking, four common mechanisms have been proposed for coexistence among ant species. These are the dominance – discovery tradeoff (Fellers 1987), the dominance – thermal tolerance tradeoff (Cerdá et al. 1997; Cerdá et al. 1998; Bestelmeyer 2000; Lessard et al. 2009), spatial segregation (Albrecht and Gotelli 2001), and partitioning of thermal niches (Torres 1984; Albrecht and Gotelli 2001). We found no support for any of these coexistence mechanisms. However, our results suggest that foraging times of species are temporally partitioned, which may promote coexistence in forest ant communities. Admittedly, however, temporal niche partitioning in this system may have also arisen for reasons entirely unrelated to interspecific interactions and coexistence.

Ant species in our system are temporally segregated, with behaviorally dominant ants occupying baits during the night and subdominant ants more prevalent during the day. Of course, further investigation is needed to be sure that these species-level interactions scale up to promote coexistence. However, evidence for the importance of temporal niches in ant communities has been found in other systems. For example, Fellers (1989) found that ant species segregated time in another eastern deciduous forest. In the Maryland forest system she worked in, *Prenolepis imparis* foraged primarily during the night in summer, while *Formica subsericea* foraged during the day. We also found *Prenolepis imparis* to be primarily nocturnal during the study periods and *Formica subsericea* and *Formica pallidefulva* to be strictly diurnal in our study system as well. Likewise, diurnally active *Formica* species in Europe have been found to shift occupation of



baits by subdominant species to the night (Vepsäläinen and Savolainen 1990). Diurnal segregation of species has also been observed in other ecosystems. In a classic study on a sand ridge in Michigan, Talbot (1946) observed diurnal variation in ant activity among three ant species. Additionally, Cerdá et al. (1997, 1998) also found variability in daily patterns of foraging among ant species in Spain. Similarly, diurnal niche partitioning was found to be important in an Oklahoma grassland, while seasonal niche partitioning was not a factor due to the clustering of species during the warmer part of the year (Albrecht and Gotelli 2001). Temperature is commonly suggested as the proximate cue for temporal niche segregation. However, considering only baiting observations occurring during the day, we found no evidence that temperature explained temporal niche segregation within the studied ant community despite the 33°C range in temperatures observed among baiting stations (though we admit that the hottest temperatures observed across this spectrum were associated with light flecks on the forest floor and, as such, were short-lived). This suggests that temperature is not the cue for foraging. We should note that these daytime temperatures did not encompass the cooler end of the nighttime temperatures, which dropped to as low as 14°C in this study, though the range of daytime temperatures did overlap the average observed nighttime temperature of 21°C. However, using only the daytime temperature data in this analysis allowed us to avoid confounding temperature and time.

If the temporal cue that ants use to decide when to forage is not temperature, it is reasonable to wonder what it might be. Recent studies suggest that light levels may play a role in determining the timing of foraging in some ant species (Narendra et al. 2010; Amor et al. 2011) while other species may switch between vision and chemical cues depending on light levels

(Beugnon and Fourcassié 1988). The bigger question, though, is what factors have favored the evolution of differences in foraging times. Perhaps competition has favored such segregation, though it is interesting that the species that are most similar to each other behaviorally (which is to say dominant *Camponotus* species) tend to forage at the same time. Another factor that could shape temporal foraging patterns may be food availability. For example, there is evidence in other systems that honeydew quality and availability may vary over the course of a day; such variation in availability of this key resource could shape daily activity patterns (Degan and Gersani 1989, Pekas et al 2011). Thus, segregation of food resources may also influence temporal foraging patterns. Alternatively, and not exclusively, phorid flies and other parasites, may limit the activity of dominant species during the day. During the study, phorid flies were frequently observed attacking *Camponotus* species during the day. Phorid flies have been found to limit daytime foraging by a species of leaf-cutter ants in Costa Rica (Orr 1992) and to cause seasonal shifts in *Pheidole titanis* toward nocturnal foraging in a dry season-deciduous thorn forest in Mexico (Feener 1988). Beyond regulating diurnal patterns, phorid flies are known to alter competitive interactions between species (LeBrun and Feener 2007).

The dominance – discovery tradeoff does not seem to mediate the coexistence of ant species in our study system. Previous studies in eastern deciduous forests on the dominance – discovery tradeoff have found support for this mechanism (Fellers 1987), while others have not (Lessard et al. 2009). However, the substantial overlap in the Bayesian confidence intervals among species indicates that a strict linear dominance hierarchy does not exist in this system. This lack of a true linear hierarchy may lessen the role of tradeoffs in functional traits in promoting coexistence. Additionally, intransitivities under certain environmental conditions or

for some resources may also lead to coexistence (Sanders and Gordon 2003). Further, our failure to find evidence of this tradeoff may be, at least in part, because one ant species (*A. rudis*) dominated bait discoveries over all other species. The ability of this single species to find food resources more efficiently than any other ant species in the system may alter the predictive power of the dominance-discovery tradeoff for other subdominant species in this system. *A. rudis* was also present in Fellers's (1987) system and was also the species most adept at discovering baits in that system. However, Fellers's system had two other commonly observed species that were fairly close to *A. rudis* in discovery ability. Interestingly, one of these species was *Temnothorax curvispinosus* (*nee Leptothorax curvispinosus*), a species which we found to be a relatively poor discoverer of food resources in our study. Variability in community composition may cause this tradeoff to be important in some systems but not in others. However, a recent review (Parr and Gibb 2011) suggests that the dominance – discovery tradeoff may not be general among ant assemblages in a variety of systems.

We also found little evidence for the dominance – thermal tolerance tradeoff in maintaining species coexistence. A dominance – thermal tolerance tradeoff may be more important in systems in which temperatures are high or low enough to limit foraging in intolerant species (Cerdá et al. 1997; Cerdá et al. 1998; Bestelmeyer 2000; Lessard et al. 2009; Wiescher et al. 2011). In hot, arid systems, the only species foraging during the hottest periods of the day are often the subordinate species (Cerdá et al. 1997; Cerdá et al. 1998), while in relatively more benign systems the reverse has been found, with subordinate ants foraging more frequently at cooler, and across a broader range of temperatures, relative to dominant ants (Lessard et al. 2009). Additionally, Lynch et al. (1980) found evidence for seasonal niche segregation in a

Maryland deciduous forest, suggesting the importance of temperature in this system on an annual timescale.

In ants, both the dominance – discovery and dominance – thermal tolerance tradeoffs are problematic as general explanations for coexistence because of the lack of an agreed upon measure of dominance (for example: Fellers 1987; LeBrun and Feener 2007). In addition, dominance hierarchies can vary based on additional variables such as temperature and time of day (Cerdá et al. 1997), resource availability (Sanders and Gordon 2003), or simply due to unmeasured or stochastic factors, making it challenging to demonstrate whether such tradeoffs promote coexistence. The uncertain status of tradeoffs as a general explanation for coexistence in ants stands in contrast to the case for many other taxa where tradeoffs seem to be common (Tilman 2011).

Finally, we did not find any evidence that ant species partition space, in contrast to the results of many studies that have shown ant species are often spatially segregated within communities (Adams 1994; Majer et al. 1994; Blüthgen et al. 2004). Many of the studies examining spatial segregation among ants have dealt with arboreal species (but see Albrecht and Gotelli 2001; Sanders et al. 2007c), while our study focused on ground-dwelling species. Additionally, temperature may mediate patterns of co-occurrence within sites, if interspecific competition and microhabitat preferences result in spatial segregation of microsites that differ in temperature (Vepsäläinen and Savolainen 1990; Wittman et al. 2010). Spatial segregation can be particularly common among dominant ants. However, we did not observe spatial segregation even among the five most dominant ant species. This result agrees with the findings of Sanders et al. (2007a) who considered dominant arboreal ant species within a tropical forest and found

them to co-occur randomly. However, Sanders et al. (2007a) did find that in a tropical agro ecosystem, all species co-occurred less frequently than expected by chance, suggesting that overall, arboreal species in that tropical system segregate space. Perhaps the result most similar to ours comes from a study of ant assemblages in the northeastern US. Gotelli and Ellison (2002) found spatial segregation to be important in ant communities at regional scales (essentially among sites separated by tens of km), but failed to find evidence for spatial segregation at local scales within sites. In our study (and in others), the lack of spatial segregation likely points to the importance of other factors in limiting competitive interactions among ant species.

In our study system the only niche-based difference among species that might facilitate coexistence was temporal niche segregation. In general, dominant ant species foraged during the night, while subdominant ants tended to recruit to baits more commonly during the day. Such segregation might result from competitive displacement, but it may simply be a result of other ecological or evolutionary processes (such as the influence of parasitism). An important implication of these results is the need to conduct more ecological research during periods traditionally less well studied (i.e., night). The vast majority of ecological studies on ants as well as other organisms are conducted during the day. However, there may be important differences in species activity during the night versus the day, and these differences may, in large part, determine the structure of some ant assemblages.

We should be clear that this study does not exhaustively test all potential mechanisms of coexistence. For example, all of the mechanisms we tested assume that interspecific competition is important in the structuring of ant communities (Hölldobler and Wilson 1990; Parr and Gibb 2010). However, intraspecific interactions also have the potential to regulate community

composition if the influence of intraspecific competition for some species is greater than the influence in interspecific competition (Shorrocks et al. 1984; Shorrocks and Sevenster 1995). While we did not explicitly quantify intraspecific interactions during this experiment, we observed interspecific interactions much more frequently than intraspecific interactions. However, the relative impacts of intraspecific competition in ants are poorly understood (but see Boulay et al. 2010). Additionally, we consider only mechanisms that involve competition for food. Competition for nest sites (Andersen 2008) and the perturbation of foraging by parasitoids (LeBrun and Feener 2007) may also play a role in structuring these communities. Even considering competition for food, another possible driver of coexistence could include segregation of particular types of food resources (Bernstein 1979; Sanders and Gordon 2003). The ants observed in this study tend to be generalist omnivores, but we do not know the extent to which their diets overlap. Additional testing, perhaps with stable isotopes, would provide more information on potential dietary differences among these species (Blüthgen et al. 2003; Tillberg et al. 2006; Fiedler et al. 2007). It should also be noted that the mechanisms examined in this study all assume that populations are in equilibrium. However, as with many other studies, we do not explicitly test this assumption. Thus, it is possible that the focal community is not at equilibrium (Siepielski and McPeck 2010). Finally, we examined temporal segregation on only a diurnal timescale, but segregation on seasonal timescales could also be important (Lynch 1981). However, while it is possible that partitioning of activity times among months or seasons throughout the year is possible, we have found little evidence of such a mechanisms in this system (Stuble, unpublished data).

Our results point not only to the possible importance of temporal niche partitioning in at least some ecosystems, but to the potential for temporal niche segregation to operate outside of the influence of temperature. Temporal segregation has also been suggested as a mechanism of coexistence in assemblages of other species as well (Carothers and Jaksic 1984; Kronfeld-Schor and Dayan 1999). However, even if temporal segregation leads to non-random temporal overlap of the activity patterns of species, temporal segregation may have evolved for other reasons (such as avoidance of parasitism) that have very little to do with interspecific competition and coexistence. Taken together, our approach of testing multiple competing hypotheses for coexistence among ant species is beginning to rule out some mechanisms while finding support for others, at least in this study system. Replicating this work in other systems, be they ant assemblages or otherwise, will provide much needed answers to one of the most vexing questions in ecology - how so many seemingly similar species coexist in local communities.

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Table I.1. Dominance (as measured by the Colley dominance matrix) and discovery abilities (residual of actual bait discovery versus expected bait discovery) for the twelve most common ants in the study system. Positive values for discovery ability indicate species that discovered more baits than expected based on worker abundance alone, while negative values indicate species that discovered fewer baits than expected based on worker abundance. Only two species, *A. rudis* and *N. faisonensis* discovered food faster than would be expected given their abundance.

Species	Dominance	Discovery Ability
<i>Aphaenogaster lamellidens</i> Mayr	0.20	n/a
<i>Aphaenogaster rudis</i> Enzmann	0.17	164.40
<i>Camponotus americanus</i> Mayr	0.89	-4.26
<i>Camponotus castaneus</i> (Latreille)	0.82	-21.55
<i>Camponotus pennsylvanicus</i> (De Geer)	0.96	-46.58
<i>Crematogaster lineolata</i> (Say)	0.94	-43.38
<i>Formica pallidefulva</i> Latreille	0.53	-16.55
<i>Formica subsericea</i> Say	0.50	-3.26
<i>Nylanderia faisonensis</i> (Forel)	0.12	6.00
<i>Prenolepis imparis</i> Emery	0.93	-18.03
<i>Tapinoma sessile</i> (Say)	0.67	n/a
<i>Temnothorax curvispinosus</i> Mayr	0.03	-16.80

Table I.2. Niche segregation for temporal and thermal niches. Bold text indicates which index of niche overlap (observed or expected) was larger.

Class	Obs. Niche Overlap	Simulated	p
<b>Thermal Niche Overlap</b>			
All	<b>0.63</b>	0.56	< 0.01
Dominant	<b>0.66</b>	0.53	< 0.01
Subdominant	<b>0.72</b>	0.63	< 0.01
Dominant v. Subdominant	<b>0.89</b>	0.76	< 0.01
<b>Temporal Niche Overlap</b>			
All	0.44	<b>0.46</b>	0.02
Dominant	<b>0.49</b>	0.45	< 0.01
Subdominant	<b>0.59</b>	0.45	< 0.01
Dominant v. Subdominant	0.67	<b>0.73</b>	< 0.01

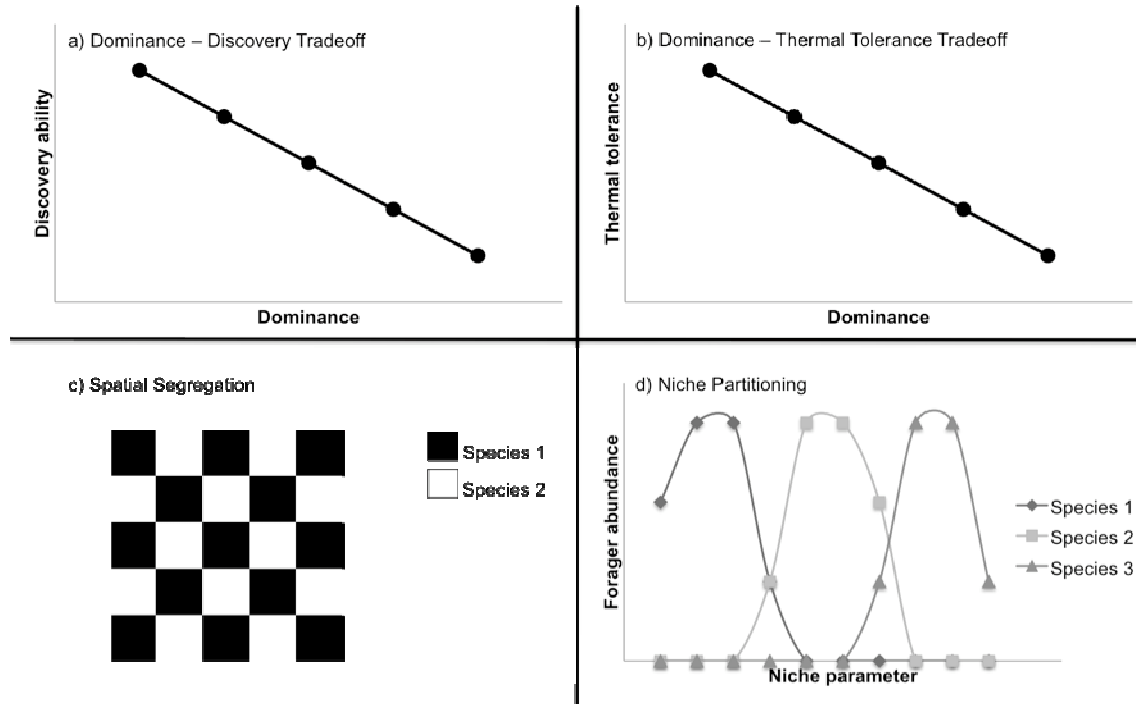


Figure I.1. Predictions of the a) dominance – discovery tradeoff, b) dominance – thermal tolerance tradeoff, c) spatial segregation, and d) niche partitioning. In figures a) and b) each point represents a species. In a) the overall pattern indicates that behaviourally dominant species tend to be less able to discover new food resources. In b) the pattern indicates that behaviourally dominant species tend to be less tolerant of temperature extremes than behaviourally subdominant species. In c) each square represents a patch of space. Alternating black and white colouring indicates that species 1 and 2 segregate this space. In figure d) each point represents the foraging effort of a given species in a given niche. We see that the three species segregate the niche space in which they forage maximally.

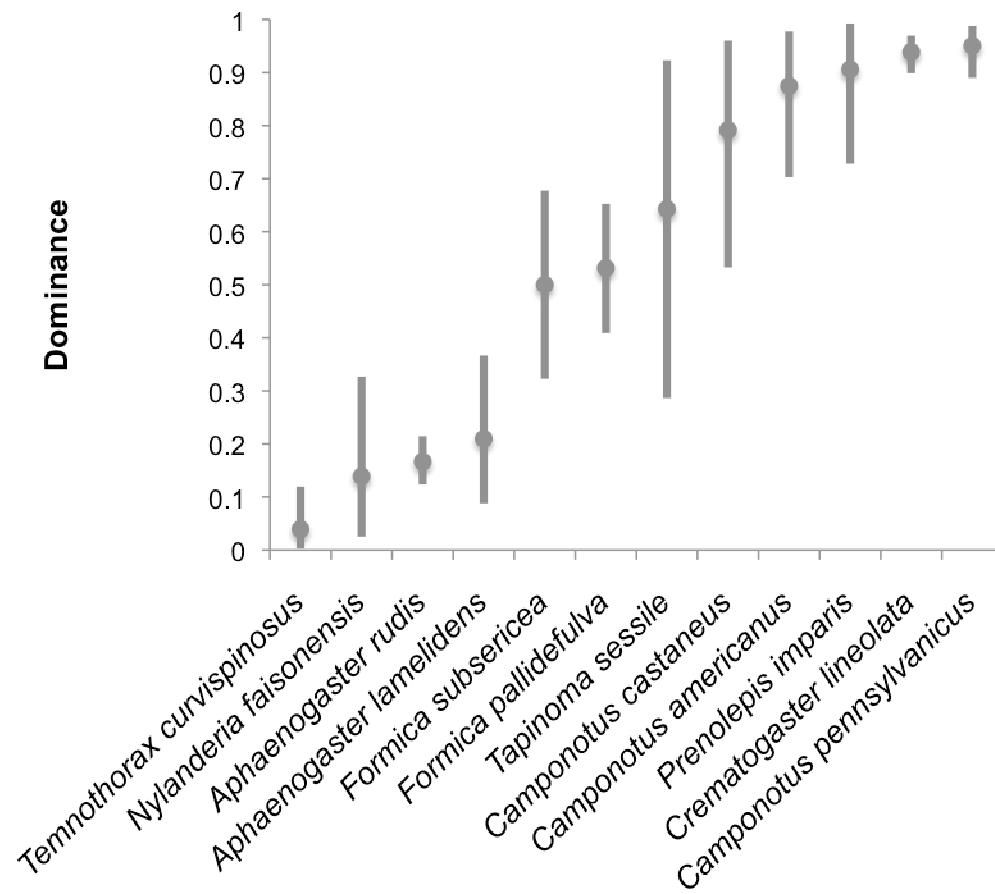


Figure I.2. Dominance (based on proportion of aggressive encounters won), plus or minus Bayesian confidence intervals.

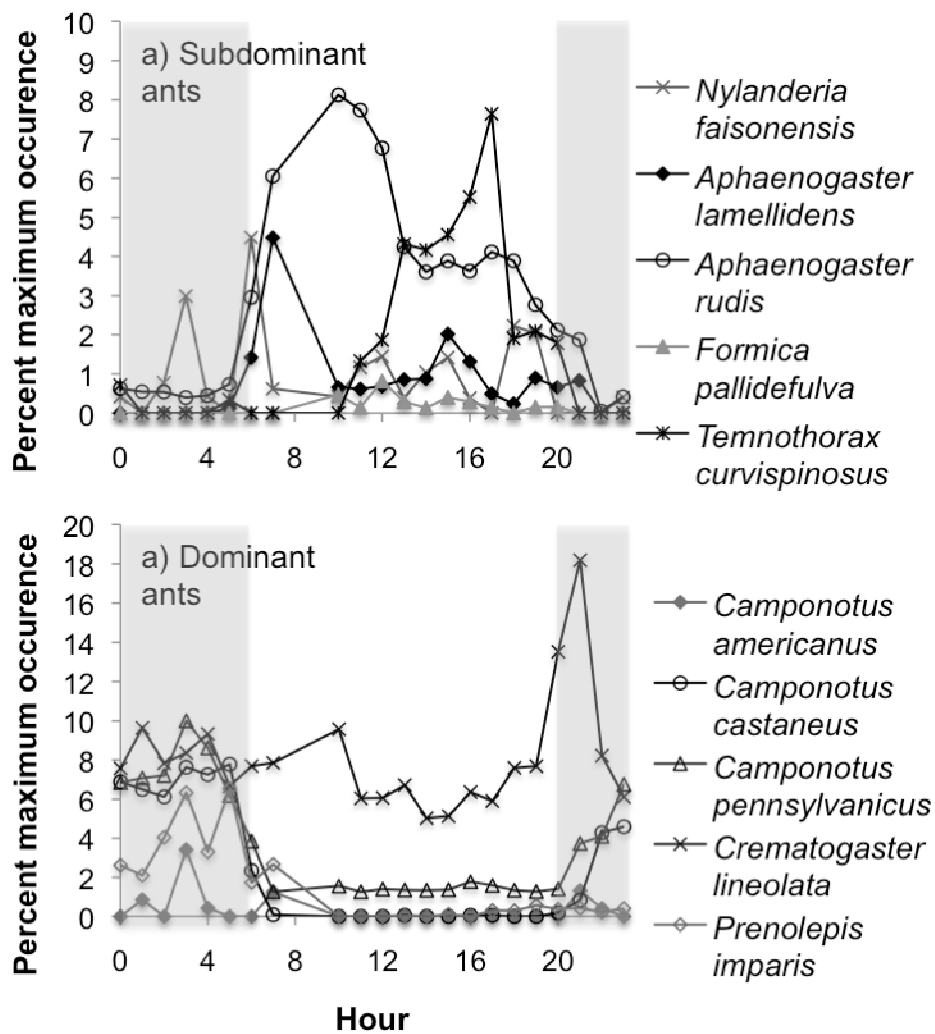


Figure I.3. Percent maximum occurrence of abundance of a) dominant ants and b) subdominant ants on baits the course of the day. Shaded areas indicate nighttime. Hour 0 indicates midnight.

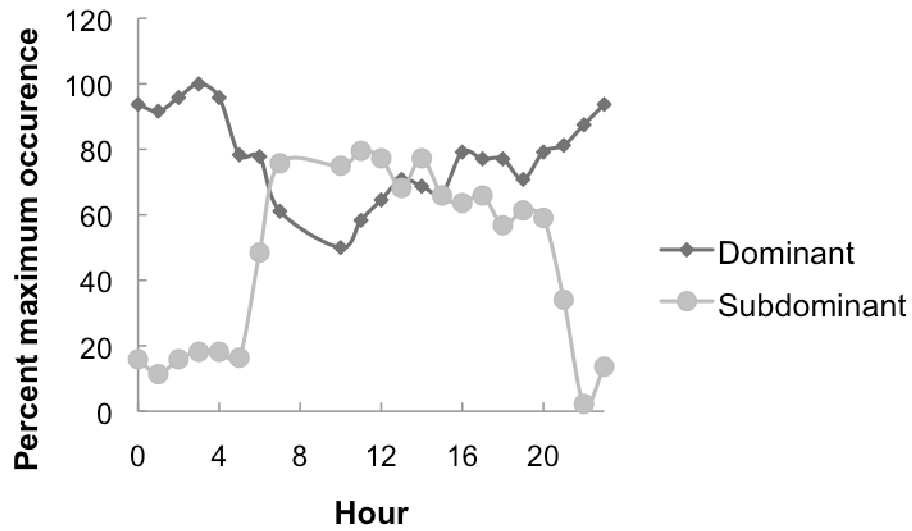


Figure I.4. Percent maximum occurrence of abundance of dominant ants (combined) and subdominant ants (combined) on baits the course of the day. Hour 0 indicates midnight.



## **CHAPTER II**

# **FORAGING BY FOREST ANTS UNDER EXPERIMENTAL CLIMATIC WARMING: A TEST AT TWO SITES**

### **Abstract**

Climatic warming is altering the behavior of individuals and the composition of communities. However, recent studies have shown that the impact of warming on ectotherms varies geographically: species at warmer sites where environmental temperatures are closer to their upper critical thermal limits are more likely to be negatively impacted by warming than are species inhabiting relatively cooler sites. We used a large-scale experimental temperature manipulation to warm intact forest ant assemblages in the field and examine the impacts of chronic warming on foraging at a southern (North Carolina) and northern (Massachusetts) site in eastern North America. We examined the influence of temperature on the abundance and recruitment of foragers as well as the number of different species observed foraging. Finally, we examined the relationship between the mean temperature at which a species was found foraging and the critical thermal maximum temperature of that species, relating functional traits to behavior. We found that forager abundance and richness were related to temperature treatment ( $\Delta^{\circ}\text{C}$ , the experimental increase in ambient temperature) at the southern site, but not the northern site. Additionally, individual species responded differently to temperature: some species foraged more under warmer conditions while others foraged less. Importantly, these species-specific responses were related to functional traits of species (at least at the Duke Forest site): species with higher critical thermal maxima had greater forager density at higher temperatures than did species with lower critical thermal maxima. Our results indicate that while climatic warming

may alter patterns of foraging activity in predictable ways, these shifts vary among species and between sites. More southerly sites and species with lower critical thermal maxima are likely to be at greater risk to ongoing climatic warming.

## **Introduction**

Climatic warming is currently shifting the phenologies and ranges of species (Parmesan and Yohe 2003, Chen et al. 2011), as well as relative abundances of species within communities (Walker et al. 2006). Warming may also alter behavior of individuals in those communities (Kearney et al. 2009, Walther 2010, Kordas et al. 2011). However, variation in the extent to which increasing temperatures alter rates of foraging likely exists among species and ecosystems. To a large degree, the vulnerability of a species to warming is mediated by the difference between thermal limits of and temperatures experienced by individuals (Kingsolver 2009, Huey et al. 2012). A change in the thermal regime is more likely to affect individuals that have smaller differences between thermal limits and environmental temperatures. This proximity to the critical thermal maximum is driven both by the thermal tolerance of the individuals within a species as well as the thermal regime of the environment, making both species identity and geographic location (or background climate) important components in predicting responses to climatic warming (Kingsolver 2009). All things being equal, this suggests that a larger effect of warming might be expected where conditions are already warm and individuals occur closer to their critical thermal maxima ( $CT_{max}$ ) (Deutsch et al. 2008). For example, tropical species typically occur at temperatures that are closer to their critical thermal maxima than do temperate species, making them more susceptible to the detrimental effects of warming (Deutsch et al.

2008, Tewksbury et al. 2008, Kingsolver 2009, Diamond et al. 2012, Huey et al. 2012). While the differences between the responses of tropical and temperate ectotherms to climate warming are apparent, the question of whether this pattern holds at higher and lower latitudes within the temperate zone is less clear.

Ants are ubiquitous in most terrestrial ecosystems and interact broadly with other species. As a consequence, changes in ant activity, as well as local and regional distributions (including those caused by temperature), can produce changes in ecosystem function including nutrient transport and seed dispersal, among other impacts (Wardle et al. 2011, Zelikova et al. 2011). Given the influence of temperature on ants, and the often substantial roles ants play in ecosystems, any change in temperature that affects ants could have cascading effects throughout terrestrial ecosystems.

In this study, we experimentally warmed ant assemblages from ambient to 5.5°C above ambient temperatures over a period of nine months (Pelini et al. 2011b). To our knowledge, this study is among the first field manipulations to experimentally warm intact animal assemblages, with replication at the southern and northern boundaries of an extensive geographic area.

Manipulations were conducted at two distinct locations in order to assess shifts in ant activity both near the northern and southern range extents of several forest ant species in eastern North America. Such experimental warming allowed us to examine explicitly the impacts of temperature on ant foraging activity, as well as how the impacts might differ at northern and southern range boundaries and among species. Here we tested four explicit predictions:

- (1) Forager abundance of individual species varies with temperature treatment ( $\Delta^{\circ}\text{C}$ ), and the responses of species to warming depend on the maximum thermal tolerances ( $\text{CT}_{\text{max}}$ ) of the species.
- (2) Species richness is lower in higher temperature ( $\Delta^{\circ}\text{C}$ ) treatments.
- (3) Warming alters the ability of ants to recruit to food resources.
- (4) Finally, the magnitude of overall and species-specific shifts in forager abundances, as well as declines in richness, in response to increased temperatures is greater at the southern site than the northern site, because southern species operate closer to their critical upper thermal limits.

## Methods

To examine the effects of chronic warming on ant foraging, we experimentally manipulated air temperature at two sites - a southern site (Duke Forest in North Carolina, USA) and a northern site (Harvard Forest in Massachusetts, USA). The experimental site at Duke Forest ( $35^{\circ} 52' 0''$  N,  $79^{\circ} 59' 45''$  W, 130 m above sea level (a.s.l.)) is in an ~80-yr old oak-hickory stand. The mean annual temperature at Duke Forest is  $15.5^{\circ}\text{C}$ , and the mean annual precipitation is 1140 mm. The experimental site at Harvard Forest ( $42^{\circ} 31' 48''$  N,  $72^{\circ} 11' 24''$  W, 300 m a.s.l.) is in a ~70-year-old oak-maple stand in the Prospect Hill Tract. The mean annual temperature at Harvard Forest is  $7.1^{\circ}\text{C}$ , and the mean annual precipitation is 1066 mm. Despite the  $8^{\circ}\text{C}$  temperature difference, Duke Forest and Harvard Forest share more than 30 ant species (Pelini et al. 2011b). Furthermore, species found at both sites tend to be at or near their northern range limits in Massachusetts and at or near their southern range limits in North Carolina.

The temperature manipulation consists of twelve open-top chambers at each site. Each chamber is an octagon that is 5m in diameter and 1.5m tall. The air within these chambers is actively warmed as described in Pelini et al. (2011b) with nine chambers set to increase ambient air temperatures by approximately 1.5 to 5.5°C above ambient temperatures, in a regression design, at half-degree steps. The three remaining chambers are controls and blow air at ambient temperatures into the chambers. Chambers have been warmed continuously since January 2010. Air temperature within the chambers is monitored continually by each of two thermistors connected to a data logger (see Pelini et al. 2011b).

Within each chamber, we placed four evenly spaced bait stations, each consisting of two resource solutions (20% sugar and 20% protein to increase the number of ant species collected). Paired sugar and protein tubes were spaced 1m apart from one another. We deployed all resource tubes at 11am to sample at a time when the majority of species were foraging. Sampling was conducted in the summer and fall at both sites.

#### RESOURCE TUBES

The 20% protein solution was made with unflavored whey protein powder (Jay Robb Enterprises, Carlsbad, CA). Both resource solutions consisted of 10 ml of solution in a 50 ml centrifuge tube containing a cotton ball to soak up the solution (Kaspari et al. 2008). Tubes were placed such that the opening was flat against the surface of the ground or leaf litter, allowing ants easy access to the resource. After two hours (at 1pm) the two resource tubes were capped and returned to the lab where all ants were identified to species. Liquid baits have been found to attract the same suite of species that are collected using other common bait types within the warming chambers (personal observation). Moreover, these techniques are being used widely to

assess resource limitation in ant communities (Kaspari and Yanoviak 2001, Kaspari et al. 2008, Kaspari et al. 2010). As with any baiting protocol, there is some chance that interference or aggressive interactions from early discoverers deter species that arrive later at the baits. However, we are not interested in quantifying competitive dominance or discovery by particular species in this particular study (but see Stuble et al. in press). Instead, we seek to document the response of the entire assemblage to resources. *Aphaenogaster rudis* and *Aphaenogaster carolinensis* were combined under the *A. rudis* complex due to their perceived morphological and ecological similarity in the field.

#### FORAGER ABUNDANCE

We calculated the number of tubes of each resource per chamber (a maximum of four) occupied by ants, as well as by each individual species, for each season and site combination. Because ants are social and live in colonies (and the colony is the unit of selection), estimating abundance is challenging. Therefore, many investigators use occupancy as an estimate of abundance (Kaspari 2001, Longino et al. 2002, Sanders et al. 2007). Here, we use bait occupancy (number or proportion of baits occupied), which is also often used as an estimate of ant abundance (Holway 1998, Ratchford et al. 2005, Wittman et al. 2010). We used ANCOVA to examine the effects of temperature treatment ( $\Delta^{\circ}\text{C}$ , which is the degrees Celsius above ambient temperature) as a continuous variable as well as site (Duke Forest or Harvard Forest), while controlling for season, and resource type (protein or sugar) (included as fixed effects), on overall and species-specific bait occupancy. Site and/or season were removed as factors from the model for species that were entirely absent from a given site or season. Models were subsequently run separately for each site to examine the relationship between temperature treatment and forager abundance in cases in

which there was a significant site-by-treatment interaction. All analyses were conducted using SAS version 9.2 and for all analyses we tested for all combinations of interactions, sequentially removing non-significant interactions. We square-root transformed data on overall ant occupancy of resource tubes to meet normality assumptions (Bolker et al. 2009). For clarity, we present untransformed data in the figures and tables. Species-specific models were not run for species observed in fewer than 8 resource tubes.

### SPECIES RICHNESS

During the summer sampling, in order to collect ants that were foraging in the chambers but not necessarily visiting the tubes, we hand sampled for five minutes in each chamber following the baiting trial. Representatives of all species seen in these five minutes were collected. We combined these data with the data from the resource tube experiment to estimate total richness for each chamber. We again used ANCOVA to examine the effects of  $\Delta^{\circ}\text{C}$  (included as a continuous variable) and site (included as a categorical variable) on species richness. We square-root transformed richness data to meet the normality assumptions of ANCOVA.

### RECRUITMENT

We estimated recruitment as the number of workers in a resource tube for each species that discovered the tube, as well as for all ant species combined. We analyzed the data using ANCOVA with  $\Delta^{\circ}\text{C}$  as a continuous variable, and site, season, and resource as discrete variables. Site and/or season were removed as factors from the model for species that were entirely absent from a given site or season. To meet assumptions of normality, overall recruitment data (data for all ants, combined) were cube-root transformed. For species-specific recruitment data, we log-transformed recruitment by both *Aphaenogaster rudis* and

*Crematogaster lineolata* (recruitment data for the remaining species did not need to be transformed).

## THERMAL TOLERANCE

Finally, we calculated the mean temperature at which each species at Duke Forest was found foraging during both the summer and the fall. To examine the relationship between thermal tolerance and foraging activity in hot chambers, we used the critical thermal maxima ( $CT_{max}$ ) determined for ants at Duke Forest in the summer of 2010 based on the temperature at which locomotive coordination was lost (the temperature was raised 2°C every 10 minutes) (Diamond et al. 2012). The rate of experimental warming and use of ramping experiments can affect estimation of thermal tolerance (Rezende et al. 2011, Terblanche et al. 2011), but we note that identical methods were used for all of the study species included here. The mean foraging temperature for each species was regressed against the  $CT_{max}$  for each species, based on worker abundances in resource tubes. We performed separate regressions for summer and fall since ambient temperatures differed between seasons and not all species were sampled in both seasons. Harvard Forest was not considered in this analysis because only three species were present in tubes, limiting our ability to conduct meaningful statistical analyses.

## Results

### FORAGER ABUNDANCE

We observed *Aphaenogaster lamellidens*, *A. rudis*, *Camponotus pennsylvanicus*, *C. lineolata*, *Formica pallidefulva*, *Nylandaria faisonensis*, *Prenolepis imparis*, and *Temnothorax curvispinosus* in resource tubes at Duke Forest and *A. rudis*, *C. pennsylvanicus* and *Myrmica*



*punctiventris* at Harvard Forest. Overall ant occupancy of resource tubes per chamber (as measured by the number of baits containing ants in a chamber) was 6× higher at Duke Forest ( $1.2 \pm 0.2$  baits chamber<sup>-1</sup>) than Harvard Forest ( $0.2 \pm 0.1$  baits chamber<sup>-1</sup>) and 6.7× higher in the summer ( $1.3 \pm 0.2$  baits chamber<sup>-1</sup>) than in the fall ( $0.2 \pm 0.1$  baits chamber<sup>-1</sup>). Overall ant occupancy did not depend on  $\Delta^\circ\text{C}$  ( $F_{1,90} = 2.42$ ,  $P = 0.12$ ) (Figure II.5), but there was a significant site by  $\Delta^\circ\text{C}$  interaction ( $F_{1,90} = 3.95$ ,  $P = 0.05$ ) such that  $\Delta^\circ\text{C}$  and ant occupancy were positively correlated at Duke Forest ( $F_{1,44} = 5.72$ ,  $P = 0.02$ ,  $R^2 = 0.58$ ), but not at Harvard Forest ( $F_{1,44} = 0.16$ ,  $P = 0.70$ ).

Responses to temperature treatment varied among species. Resource tube occupancy by *C. pennsylvanicus* and *A. rudis* were unrelated to  $\Delta^\circ\text{C}$  while *P. imparis* was negatively associated with  $\Delta^\circ\text{C}$ , and *F. pallidefulva* and *C. lineolata* were positively associated with  $\Delta^\circ\text{C}$  (Table II.3). Only *A. rudis* and *C. pennsylvanicus* were present at both sites in numbers great enough to allow for analysis of between-site variation, though neither species differed significantly in forager abundance between the two sites or among temperature treatments (Table II.3).

## SPECIES RICHNESS

Species richness of actively foraging ants was 3.2× higher at Duke Forest ( $4.3 \pm 0.5$  species chamber<sup>-1</sup>) than at Harvard Forest ( $1.3 \pm 0.5$  species chamber<sup>-1</sup>). Temperature treatment ( $\Delta^\circ\text{C}$ ) was not related to species richness ( $F_{1,20} = 1.03$ ,  $P = 0.31$ ) (Figure II.6), but there was a significant temperature × site interaction ( $F_{1,20} = 2.55$ ,  $P = 0.02$ ) such that species richness at Duke Forest was marginally positively correlated with  $\Delta^\circ\text{C}$  ( $F_{1,10} = 4.04$ ,  $P = 0.07$ ), while there was no relationship between richness and  $\Delta^\circ\text{C}$  at the Harvard Forest site ( $F_{1,10} = 2.82$ ,  $P = 0.12$ ).

## RECRUITMENT

Recruitment (number of ants in a resource tube assuming that tube had been discovered) was not related to  $\Delta^{\circ}\text{C}$  after controlling for the effects of site, season, and resource type (total ants on baits regardless of species identity) ( $F_{1,31} = 1.51$ ,  $P = 0.23$ ). This was also true when considering recruitment for individual species (Table II.4), which showed no response to variation in  $\Delta^{\circ}\text{C}$ .

## THERMAL TOLERANCE

At Duke Forest, species with a higher  $\text{CT}_{\text{max}}$  tended to be more abundant in warmer chambers than did those species with a lower  $\text{CT}_{\text{max}}$ .  $\text{CT}_{\text{max}}$  across species was significantly correlated with the mean temperature at which individuals were found foraging in both the summer ( $F_{1,4} = 7.73$ ,  $P = 0.0498$ ,  $R^2 = 0.66$ ) and fall ( $F_{1,4} = 12.76$ ,  $P = 0.02$ ,  $R^2 = 0.76$ ) at Duke Forest (Figure II.7). There were six species present in both the summer and fall at Duke Forest, with four species present in both seasons: *A. rudis*, *C. lineolata*, *N. faisonensis*, and *T. curvispinosus*. In the summer *C. pennsylvanicus* and *F. pallidefulva* were also present, while *A. lamellidens* and *P. imparis* were present in the fall. This analysis included ants from only Duke Forest because the Harvard Forest site was too depauperate to perform meaningful analyses.

## Discussion

Climatic warming continues to change the structure and function of communities (Parmesan 1996, Thomas et al. 2006, Kardol et al. 2010, Walther 2010, Sheik et al. 2011). However, the magnitude and direction of these changes may vary by region (Kingsolver 2009, Pelini et al. 2011a) and depend on community composition (Williams et al. 2008). By experimentally manipulating temperatures of intact ant assemblages, we found evidence for shifts in foraging activity of ant species in line with predictions based on their thermal tolerances at the southern

site. Importantly, however, overall patterns of forager abundance and richness to temperature treatment ( $\Delta^{\circ}\text{C}$ ) varied between the northern and southern sites, with greater impacts at the more southerly site.

These results are not entirely surprising as temperature clearly influences foraging in ants and many other ectotherms (Cerdá et al. 1997, Cerdá et al. 1998, Ruano et al. 2000, Hurlburt et al. 2008) (Traniello et al. 1984). More basically, temperature, at least for some species, can also regulate the onset and cessation of foraging, whether daily (Talbot 1943), or seasonally (Sanders 1972, Markin et al. 1974). These factors may ultimately influence both the likelihood that an ant will be able to find food resources under warmed conditions, and also the extent to which a species will recruit to that food resource once it is discovered. However, the effects of temperature increases on foraging behavior and activity are likely driven, in part, by species-specific thermal tolerances. As such, thermal tolerance can ultimately regulate ant foraging activity in a warmer climate and determine the susceptibility of ants to climatic warming (Diamond et al. 2012). In addition, other temperature-related factors, including the rapid breakdown of pheromones at high temperatures and running speeds, likely play a role in regulating the impacts of temperature on ant foraging activity (Hurlburt et al. 2008, van Oudenhove et al. 2011, van Oudenhove et al. 2012).

The effect of temperature on forager abundances varied between sites: temperature was significantly and positively associated with forager abundance at the southern site (Duke Forest), but not at the northern site (Harvard Forest), indicating the importance of geographic location in determining the impact of warming on communities. The effects of geographic location are similarly important among populations of spiders. Spiders from warmer locations are more heat-

tolerant than those from cooler locations, leading to variability in foraging behavior (Barton 2011). In ants at least, such geographic variation in response to temperatures likely scales up to influence geographic distributions of species in a warmed world. For instance, Fitzpatrick et al. (2011) suggested that ant assemblages from cooler regions of North America are likely to be more resilient to climatic warming than are assemblages from warmer regions, due, in part, to the smaller range sizes of southern populations.

Further, we found that the effects of warming on species richness varied between sites: species richness was positively correlated with warmer temperatures ( $\Delta^{\circ}\text{C}$ ) at the southern site but not at the northern site. This increase in richness at the southern site may be the result of combined effects in increased colonization of sites by thermophilic species along with an increase in worker abundance and activity by species already present. Though the overall trend at the southern site was an increase in richness with increasing temperatures, the abundances of some species declined with warming, while the abundance of others increased (Pelini et al. 2011a). In particular, foraging by two species (*C. pennsylvanicus* and *A. rudis*) did not depend on temperature, foraging by two others (*F. pallidefulva* and *C. lineolata*) increased with temperature, and foraging by one (*P. imparis*) decreased with temperature. Notably, the two species that responded positively to temperature (*F. pallidefulva* and *C. lineolata*) were observed only at the southern site in this study. However, this was also true of the only species found to respond negatively to warming (*P. imparis*, called the “winter ant” because of its propensity to forage in cooler seasons) which was observed only at Duke Forest and only during the fall sampling event. These species-specific responses are congruent with what we know about the annual and diurnal foraging patterns in these species. Foraging by *P. imparis* is thought to be

driven primarily by temperature with an optimal temperature range between 7 and 16°C (Talbot 1943). *Formica pallidefulva* (formerly *F. incerta*) forages almost exclusively during the day, a habit that Talbot (1946) found to be driven by temperature. Talbot never found *F. pallidefulva* foraging below 15.5°C, and suggested that its optimal foraging temperature ranged from 29.5°C and 35°C, though she also observed the species foraging at temperatures exceeding 37.5°C. Likewise, *C. lineolata* responds positively to increased temperature, occurring at higher densities under experimentally warmed conditions (Pelini et al. 2011a). Such variability in responses to warming among ants may lead to shifts in the biogeographic distributions of ant functional traits, though, inversely, this variability in thermal preferences could also result from differences in biogeographic distributions (Fitzpatrick et al. 2011).

Thermal physiology was a strong predictor of the foraging responses of species to experimental climatic warming at the southern site. Species with higher thermal tolerances exhibited more foraging activity in warmer chambers at Duke Forest, and this pattern held during both the summer and fall. This disparate response to temperature across species points to the potential utility of  $CT_{max}$  in determining species-specific foraging responses to warming. Indeed,  $CT_{max}$  is a strong predictor of cumulative ant activity density in this system: species with higher  $CT_{max}$  are generally more abundant in pitfall traps (Diamond et al., in press). Unfortunately, there were not enough actively foraging species collected in this study to conduct similar analyses for the Harvard Forest site. However, we note that  $CT_{max}$  values for ectotherms tend to be fairly constant across latitudes, likely placing the environmental conditions experienced by northern populations farther from their upper thermal limits (Sunday et al. 2011, Hoffmann et al. 2012). Our results suggest that understanding a key physiological trait can illuminate species-specific responses to

climatic warming and potentially lead to the development of robust predictions about the response of biodiversity to warming. In general, there is growing evidence that suggests an important role for physiological traits in better informing species' responses to global climatic warming (Kingsolver 2009, Barton 2011), and our study provides additional support for this relationship. Importantly, our study also establishes a critical link between thermal physiology and species-specific responses to large-scale experimental climatic warming.

These changes in forager abundance did not arise because of variation in recruitment ability with warming because, if a species found a bait, recruitment was essentially equivalent across all temperatures. It is also important to note that the  $CT_{max}$  of a species and its abundance in chambers tracked temperature in both the summer, as well as the fall when temperatures were far from  $CT_{max}$ . This suggests that the relationship between  $CT_{max}$  and forager abundance across temperature manipulations may be driven by overall shifts in forager abundances in the warmed chambers rather than behavioral shifts in recruitment ability related to temperature. If this were the case we would expect to see both altered recruitment ability as a function of temperature as well as a reduced importance of  $CT_{max}$  in driving foraging patterns in the warmed chambers when temperature conditions are far from  $CT_{max}$ .

The experimental warming chambers in which this study was conducted are completely open at the top and elevated approximately 2.5 cm from the soil surface at the bottom, meaning that it is possible for the ants we see foraging in the chambers to come from colonies that are outside the chambers (Moise and Henry 2010). While we cannot be certain where the ants are nesting in this study, if the ants we see in the chambers actually came from outside of the chambers, our results still demonstrate that foraging activity depends on temperature. But, we suspect that it is unlikely

that many of the ants we see foraging in the chambers come from colonies outside the chambers because the foraging ranges of the most commonly observed species are generally less than 1m (Pudlo et al. 1980, personal observation).

When temperature altered foraging activity, its effects tended to be present at only the southern site (Duke Forest). This is consistent with the findings of Pelini et al. (2011a) who also showed warming to have less of an impact on species richness and foraging activity at Harvard Forest than Duke Forest. However, it is notable that this trend remains despite the greater magnitude of warming in this study (up to +5°C) relative to that in the Pelini et al. (2011a) study (+1°C which was conducted with passive warming chambers). The lack of an effect of warming at the northern site may point to the resilience of ants at the northern end of their range to fairly substantial warming. More generally, the results of both the Pelini et al. (2011a) study and ours are consistent with other reports suggesting that warming may be more detrimental at lower latitudes (Deutsch et al. 2008, Tewksbury et al. 2008, Kingsolver 2009, Huey et al. 2012). While these studies typically involve comparisons of tropical and non-tropical regions, our study detected significant site-level differences in responses to warming when comparing communities along a latitudinal gradient entirely within the temperate zone. This may suggest that, along with tropical regions, the flora and fauna of low-latitude portions of the temperate zone may also be at increased risk as a result of climate change.

Our study focuses on short-term responses to warming. However, over more generations, the ant populations in our experimental arrays might also adapt to chronic warming (Davis et al. 2005, Skelly et al. 2007, Hof et al. 2011, Hoffmann and Sugò 2011). While the genetic architecture of thermal preference and performance is generally unknown, recent work suggests that adaptation

– particularly via foraging responses – may be constrained in ectotherms such that they will not be able to evolve fast enough to cope with climatic warming (Davis et al. 2005, Sinervo et al. 2010). Thus, we might expect that, in our experimental warming array, ant species that have a low  $CT_{max}$  will not experience sufficient evolutionary change to be able to inhabit the warmest chambers. If indeed limited adaptive ability is a widespread pattern, ants and ectotherms more generally will be reliant on acclimation, behavior, and dispersal responses to warming, i.e. the shorter-term types of responses captured by the warming experiment described here.

In summary, under experimentally warmed conditions, we found that warming had a greater impact at the southern range limit than at the northern range limit, and, at least at the southern site, species with higher  $CT_{max}$  foraged more heavily at warmer temperatures than did species with lower  $CT_{max}$ . The altered levels of foraging activity as a result of warming may have important implications for both species persistence as well as ecosystem functioning. Taken together, our results indicate that predicting the effects of temperature on the structure and dynamics of communities requires a more nuanced understanding of the behavior and thermal physiology of individual taxa, and how the responses of those taxa vary geographically.

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Table II.3. ANCOVA table of ant abundance as measure by the number of resource tubes containing a worker of a given species. When species were only observed during on season or at one site, season and / or site were not included as factors. Interactions were removed from the model when non-significant. Treatment refers to the experimental warming treatment.

<b>Species</b>	<b>Variable</b>	<b>d.f.</b>	<b>F</b>	<b>p</b>
<i>Aphaenogaster rudis</i> Enzmann	treatment	1,91	0.56	0.46
	resource	1,91	8.32	<0.01
	site	1,91	0.07	0.79
	season	1,91	5.57	0.02
<i>Camponotus pennsylvanicus</i> (De Geer)	treatment	1,91	0.02	0.87
	resource	1,91	9.36	<0.01
	site	1,91	2.89	0.09
	season	1,91	9.36	<0.01
<i>Crematogaster lineolata</i> (Say)	treatment	1,44	4.33	0.04
	resource	1,44	0.87	0.36
	season	1,44	24.6 4	<0.01
<i>Formica pallidefulva</i> Latreille	treatment	1,21	4.50	0.05
	resource	1,21	0.67	0.42
<i>Prenolepis imparis</i> Emery			13.3	
	treatment	1,21	0	<0.01
	resource	1,21	0.48	0.49



Table II.4. ANCOVA table of recruitment ability (number of workers if a species was present on a bait). Treatment refers to the experimental warming treatment.

<b>Species</b>	<b>Factor</b>	<b>d.f.</b>	<b>F</b>	<b>p</b>
<i>Aphaenogaster rudis</i>	treatment	1,11	0.76	0.40
	resource	1,11	0.85	0.38
	site	1,11	0.03	0.86
	season	1,11	2.32	0.16
<i>Camponotus pennsylvanicus</i>	treatment	1,5	1.02	0.34
	resource	1,5	0.04	0.85
<i>Crematogaster lineolata</i>	treatment	1,14	1.21	0.29
	resource	1,14	4.82	0.05
	season	1,14	6.57	0.02
<i>Formica pallidefulva</i>	treatment	1,2	1.91	0.3
	resource	1,2	0.13	0.75
<i>Prenolepis imparis</i>	treatment	1,11	0.43	0.52
	resource	1,11	17.95	<0.01

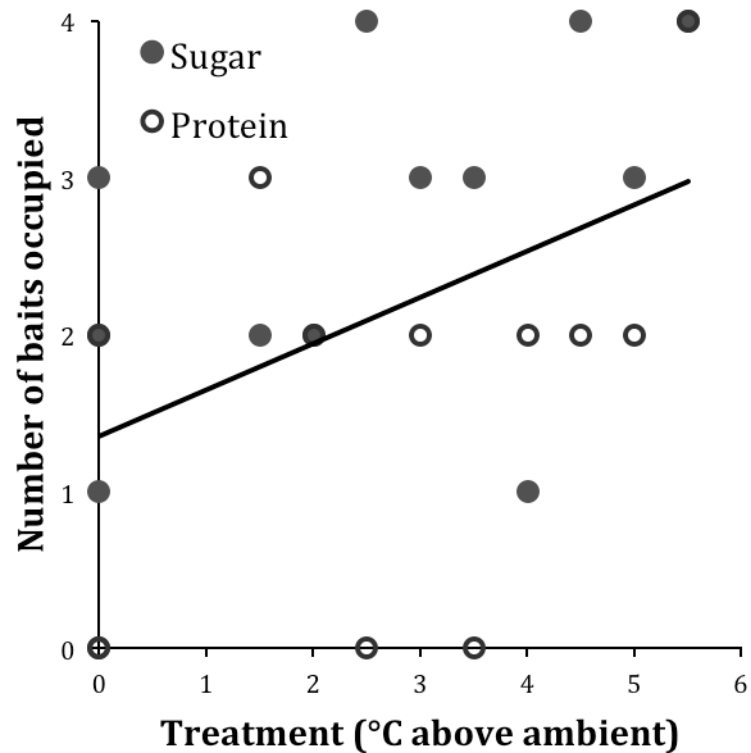


Figure II.5. Ant occupation of protein and sugar baits as a function of temperature treatment.

There is no significant effect of temperature treatment on ant abundance, though there was a significant site  $\times$  treatment interaction. Only data from Duke Forest in the summer are shown, showing a positive relationship between bait occupancy and temperature treatment. The line is the best-fit line through all of the points, regardless of bait type.

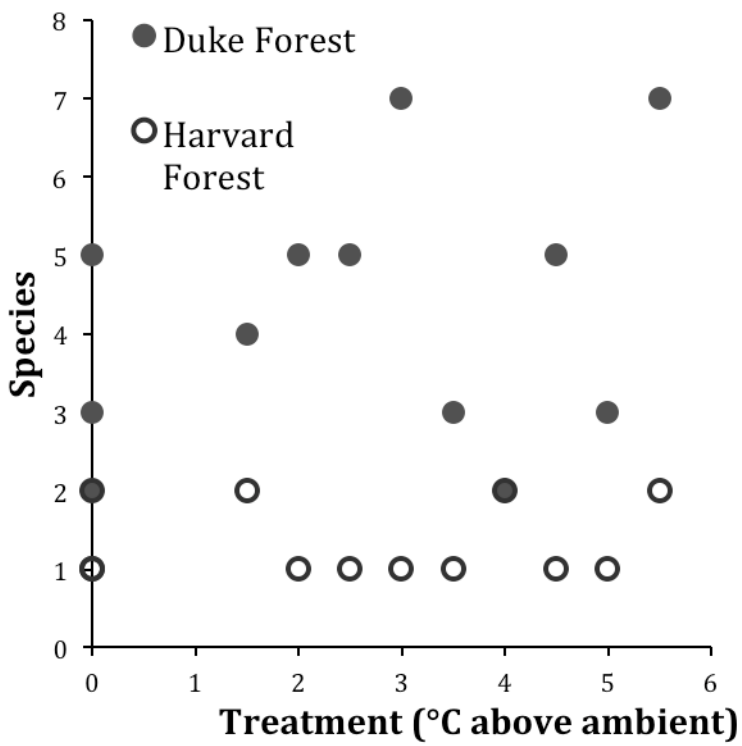


Figure II.6. Species richness as a function of temperature treatment. There is no relationship between temperature treatment and richness at either site.

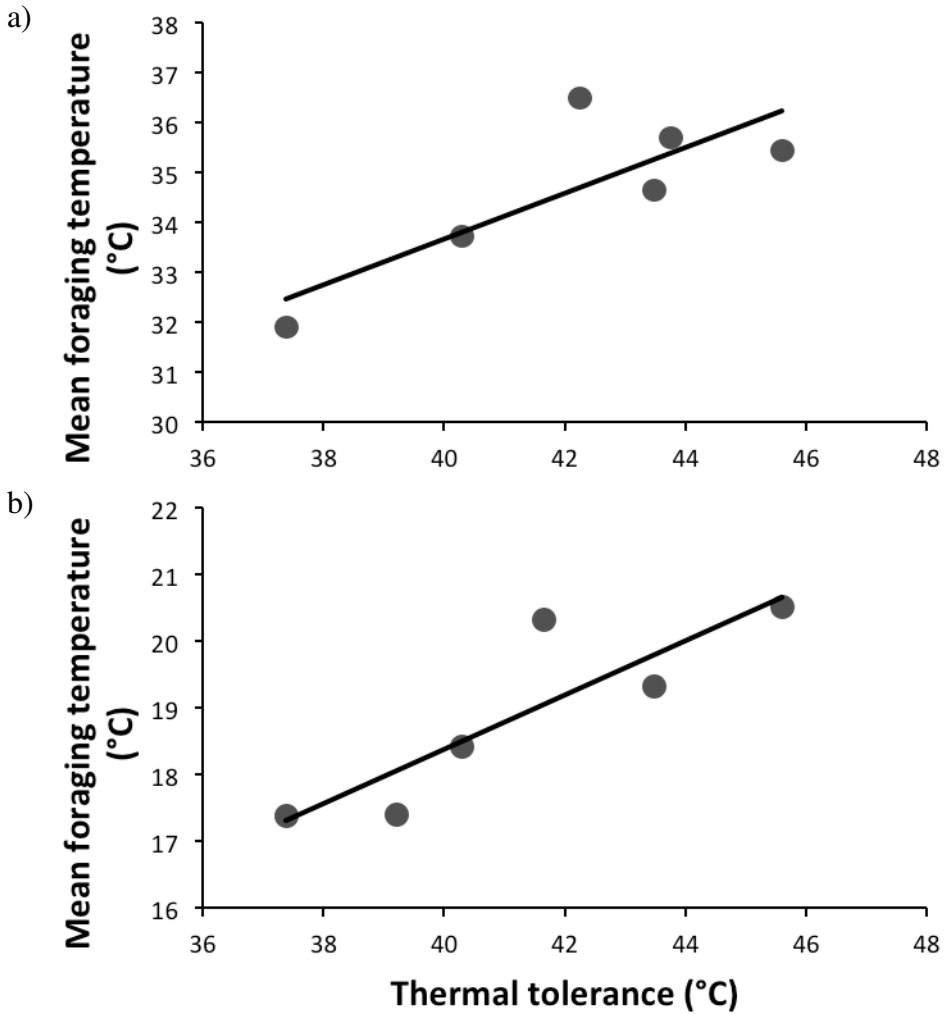


Figure II.7. Mean temperature at which a species was observed foraging as a function of the critical thermal maximum in a) the summer and b) the fall at Duke Forest. Each point represents a species and the line represents the best-fit linear regression.

# CHAPTER III

## ANT-MEDIATED SEED DISPERSAL IS RESISTANT TO EXTREME EXPERIMENTAL WARMING

### Abstract

Climate change affects communities both directly and indirectly via changes in interspecific interactions. One such interaction that may be altered is the ant-plant seed dispersal mutualism common in deciduous forests of the eastern US. As climatic warming alters the abundance and activity levels of ants in these systems, the potential exists for shifts in rates of ant-mediated seed removal. We used an experimental temperature manipulation at two sites in the eastern US (Harvard Forest in Massachusetts and Duke Forest in North Carolina) to examine the potential impacts of climatic warming on overall rates of seed dispersal (using *Asarum canadense* seeds) as well as species-specific rates of seed dispersal at the Duke Forest site. We also examined the relationship between ant critical thermal maxima ( $CT_{max}$ ) and the mean seed removal temperature for each ant species. We found that seed removal rates did not change as a result of experimental warming at either study site, nor were there any changes in species-specific rates of seed dispersal. There was, however, a positive relationship between  $CT_{max}$  and mean seed removal temperature, whereby species with higher  $CT_{max}$  removed more seeds at hotter temperatures. Temperature at these sites was influenced by experimental warming as well as diurnal and day-to-day fluctuations in temperature. Taken together, our results suggest that while temperature may play a role in regulating seed removal by ants, the ant-plant seed dispersal mutualism may be fairly resilient to climatic warming.

## Introduction

Understanding how organisms will respond to ongoing changes in climate, leading to subsequent changes in key ecological processes, is essential to predict the structure and function of ecosystems in the future. For example, the alteration of interspecific interactions is one important mechanism by which climate change may ultimately alter the structure and function of ecosystems (Tylianakis et al. 2008, van der Putten et al. 2010, Walther 2010, Harley 2011). The majority of studies on the effects of climate change on interspecific interactions have focused on negative interactions, such as competition (Suttle et al. 2007), predator-prey interactions (Rothley and Dutton 2006, Harley 2011), and herbivory (Bale et al. 2002). Though relatively rare, empirical studies of the effects of climate on interactions have found that climate change can alter the nature of interspecific interactions through a variety of mechanisms including, but not limited to, altered abundance and fitness levels of key species (Suttle et al. 2007), shifts in phenology (Both et al. 2009), and species range shifts (Harley 2011), all of which can decouple interspecific interactions by altering the amount to contact species have with one another and the ways in which they interact.

Though most studies linking climate change and interactions have focused on negative interactions (as have most ecological studies more generally), mutualisms also play a critical role in structuring communities and maintaining biodiversity (Callaway 1995, Stachowicz 2001). Mutualisms including plant-pollinator interactions and mycorrhizal interactions have been altered by climate change (Parrent et al. 2006, Memmott et al. 2007, Hoover et al. 2012). However, the influence of climate change on other types of positive interactions is not as well studied.

One such mutualism that may be altered by climate change is the ant-plant seed dispersal mutualism, which includes hundreds of ant species and thousands of plant species around the world (Beattie and Hughes 2002; Gove et al 2007; Rico-Gray and Oliviera 2007, Lengyel et al. 2010). Plants benefit from dispersal away from the parent plant because of reduced seed mortality that arises from density-dependent effects (Janzen 1970). Additionally, directed dispersal toward ant nests can often lead to seeds being deposited in a nutrient-enriched microsite for seedling establishment that also offers protection from predation and fire (Culver and Beattie 1978; Heithaus 1981, Beattie 1985).

In deciduous forests of the United States approximately thirty percent of understory herb species might be ant-dispersed (Beattie and Culver 1981), and a proposed keystone ant species, *Aphaenogaster rudis*, is responsible for upwards of 90% of ant-mediated seed dispersal (Ness et al. 2009; Zelikova et al. 2008). Such specialization in interactions can make interaction networks more vulnerable to disruption (Aizen et al. 2012). For seed-dispersal mutualisms then, myrmecorous plant species that rely on a single ant species for seed dispersal may be at increased risk for disruption by ongoing climatic change (Pelini et al. 2011a, Warren et al. 2011). However, despite the importance and ubiquity of ant-plant seed-dispersal mutualisms, experiments examining the consequences of climatic change on ant plant-seed dispersal mutualisms are rare (but see Pelini et al. 2011a).

To examine the potential for climate change to alter seed dispersal mutualisms in deciduous forests, we experimentally manipulated temperature at two forested sites in the eastern United States (North Carolina and Massachusetts) and examined overall rates of seed dispersal as a function of temperature. At the North Carolina site, we also examined species-specific rates

of seed dispersal. We test two specific predictions:

Prediction 1: Rates of seed removal by ants would decrease as a result of experimental warming at both sites, but the effect of warming would be stronger at the southern site than at the northern site. Our prediction was based on the fact that many species at the southern site exist closer to their upper thermal limits, putting them at increased risk for declines in abundance and/or activity with warming (Deutsch et al. 2008, Diamond et al. 2012, Huey et al. 2012)(Diamond et al. in press) Additionally, we have observed greater shifts in ant activity at the southern site than at the northern site (Diamond et al in press, Stuble et al in review).

Prediction 2: Species with higher critical thermal maxima ( $CT_{max}$ ) would remove seeds more readily under warmer conditions as compared to species with lower critical thermal maxima as these species are more tolerant of higher temperatures and have been found to be more active as temperatures increase (Stuble et al. in review).

## **Methods**

### **Site description**

This experiment was conducted at Duke Forest in North Carolina and Harvard Forest in Massachusetts in order to examine the potential impacts of climate change on seed dispersal mutualisms near the southern extent and northern extent of the ranges of several ant species. The Duke Forest site consists of a closed-canopy oak-hickory (*Quercus spp.*-*Carya spp.*) forest with a mean annual temperature of 15.5°C and approximately 1140 mm of precipitation annually. The Harvard Forest site is in a closed-canopy oak-maple (*Quercus spp.*-*Acer spp.*) forest with a mean annual temperature of 7.1°C and 1066 mm of precipitation a year. The two sites share ~30 ant



species, with the North Carolina site near the southern range edge and the Massachusetts site near the northern range edge for many of these species (Pelini et al. 2011b). The most abundant ant species at both sites is *A. rudis*. *Crematogaster lineolata* can be quite abundant at the southern site in warmer months while *Formica subsericea* and *Camponotus pennsylvanicus* are the two next most abundant ant species at the Harvard Forest site. For the purpose of this study we are lumping *Aphaenogaster rudis* and *Aphaenogaster carolinensis* into the *A. rudis* complex due to the difficulty of identifying these two closely related species in the field.

At each site, there are twelve experimental open-top warming chambers (Figure III.8). Each chamber is 5 m in diameter and 1.2 m tall with a 2-3 cm gap at the bottom to allow ants and other organisms to move in and out. Nine chambers at each site are warmed from 1.5°C to 5.5°C in 0.5°C steps using air warmed by hydronic radiators, while the three control chambers blow air at ambient temperatures into the plots (see Pelini et al. (2011b) for a detailed description of the chambers). Treatments have been maintained continuously since January of 2010.

### **Seed removal**

To assess the impact of temperature on rates of seed dispersal, we haphazardly positioned one seed cache in each of the 12 chambers at Duke Forest and Harvard Forest. Each cache contained 20 seeds of the myrmecochorous species *Asarum canadense*. *Asarum canadense* ranges along the east coast of the United States from New Brunswick, Canada to North Carolina (Cain and Damman 1997) and produces seeds that are similar in mass to many other myrmecochorous plant species (Michaels et al. 1988). Seeds used in the trials at Duke Forest were collected at North Carolina State University's Schenck Forest in Raleigh, North Carolina

on May 11, 2011 and those used in the Harvard Forest trials were collected from Mt. Toby in Massachusetts on June 8, 2011. We covered each seed cache with a mesh cage to allow ants to access the seeds while preventing access by rodents. Caches were left out for one hour, after which time the number of seeds remaining in the cache was counted and any remaining seeds were removed from the chamber. A total of ten trials were conducted at Duke Forest between May 12 and May 25, 2011, with five trials conducted during the day (between 0900 and 1900) and five during the night (between 2100 and 0500). Another five trials were conducted at Harvard Forest between June 16 and June 30, 2011; three during the day and two at night. These dates corresponded with the time period during which the seeds were naturally released at each geographic location.

We calculated the average seed dispersal rate (number of seeds removed in an hour) for each chamber at each site. We used ANCOVA to examine differences in seed dispersal rates as a function of temperature treatment (which we refer to as  $\Delta^{\circ}\text{C}$ , included as a continuous variable) and site. The number of seeds removed per hour was square root transformed to meet assumptions of normality. All statistics were performed in SAS, version 9.2.

To determine the ant species responsible for removing the seeds, we continuously observed caches of 10 *A. canadense* seeds within the chambers at Duke Forest for one hour, or until all seeds were removed. Four seed removal observations were conducted in each chamber: two during the day and two during the night. We recorded the identity of the ant species removing the seeds. At the beginning of each observation, we took four ground surface temperature measurement using a handheld infrared thermometer (Raytek® Raynger ST), one at each corner of the seed cache, which were averaged together. These temperature readings

provided us with estimates of ground-surface temperature conditions in the immediate vicinity of the seeds. We calculated the percentage of seeds removed by each species overall, as well as separately for day and night. We also calculated the mean number of seeds removed by each species in each chamber across all trials.

We used linear regressions to examine differences in seed dispersal rates across temperature treatments for each ant species. (We examined several polynomial regressions, but found none of them to be a better fit than simple linear regressions.) Mean numbers of seeds removed were log transformed to meet assumptions of normality for *A. rudis* and *C. lineolata*.

Finally, we calculated the average ground surface temperature (based on temperatures collected with the infrared thermometer) at which each species removed seeds across all treatments and times. We then examined the relationship between the average temperature at which a species removed seeds and the  $CT_{max}$  of that species (as calculated by Diamond et al., 2012) across all species observed removing seeds in the system. *Aphaneogaster lamellidens* was excluded from this analysis as it was only observed removing seeds from two seed caches and was an outlier (as indicated by a plot of residuals by predicted values).

## Results and Discussion

In contradiction to prediction 1, seed removal rate did not depend on temperature treatment and did not vary between sites ( $F_{2,21} = 0.93$ ,  $p = 0.41$ ) (Figure III.9). Ants removed an average of 23% of seeds (4.6 out of 20 seeds) per hour across temperature treatments and sites. Further, the effect (or rather lack thereof) of warming on seed removal was independent of geography, with no response to warming observed at two distinct geographic locations. At the

Duke Forest site, seven ant species were observed removing seeds across a range of ground surface temperatures from 17°C to 30°C. *Aphaenogaster rudis* was the most common seed disperser, removing approximately 45.5% of seeds (Table III.6). However, there was no relationship between the rate of seed dispersal by *A. rudis* and temperature treatment (Table III.5). Further, with the exception of *C. lineolata*, which showed a marginally significant increase of approximately 0.1 seeds removed per degree of warming, seed removal did not respond significantly to temperature treatment for any ant species (Table III.5). Thus, despite previously observed shifts in foraging under experimentally warmed conditions (Pelini et al. 2011a), warming did not seem to affect the aspects of the seed dispersal mutualisms we studied in this system.

Our results suggest that the ant-plant seed dispersal mutualism common in the eastern US might be resistant to increases in temperature. *Aphaenogaster rudis* is considered to be a keystone mutualist in this system as it has been found to be responsible for the majority of ant-mediated seed dispersal in a range of deciduous forests in the eastern US (Zelikova et al. 2008, Ness et al. 2009). This species has not been found to decline either in abundance (Pelini et al. 2011a) or foraging activity (Stuble et al. in review) as a result of experimental warming at either study site. The resistance of this important seed dispersing species to warming may play a major role in promoting the stability of ant-plant seed dispersal mutualisms to warming. As opposed to warming, the invasion of the exotic ant species *Pachycondyla chinensis* into deciduous forests of the eastern US has been found to both drastically decrease abundances of *A. rudis* as well as decrease rates of ant-mediated seed removal in invaded areas (Rodriguez-Cabal et al. 2012),

providing further evidence regarding the importance to *A. rudis* in maintaining ant-mediated seed dispersal mutualisms.

However, it is important to note that more than half of the seed removals observed in this study were conducted by species other than *A. rudis*. Foraging activity in several of these species, including *C. lineolata* and *Formica pallidefulva*, has been found to shift with warming (Stuble et al. in review); though individually these species do not account for a substantial percentage of seed removals in this study and we did not observe significant shifts in seed removal rates as a result of warming in any of these species. Having multiple ant species interacting in the ant-plant seed dispersal mutualism may serve as a stabilizing effect on the ant-plant seed dispersal mutualism, potentially conferring additional resistance on the mutualism. While *A. rudis* has been suggested as a keystone mutualist for the ant-plant seed dispersal mutualism in the eastern US (Ness and Morin 2007, Zelikova et al. 2008), we found the species to disperse less than half of the seeds observed at the Duke Forest site. Further, six ant species were observed removing seeds in addition to *A. rudis*. The diversity of the ant-plant seed dispersal mutualism at the Duke Forest site along with the variable effects of warming among these ant species (Stuble et al. in review) may confer some degree of resistance on this mutualism. The desirability of elaiosomes as a food source for ants (Reifenrath et al. 2012) also has the potential to make the ant-seed dispersal mutualism more resistant to climate change if their desirability is enough to cause ants to forage even in suboptimal conditions.

In addition to the resistance of *A. rudis* to warming, another factor possibly strengthening the resistance of the ant-plant seed dispersal mutualism to warming may be the timing of ant-mediated seed dispersal within deciduous forests of the eastern US. Ant-dispersed seeds within

these forests are primarily dispersed in the spring (Thompson 1981). *Asarum canadense*, the plant species used in this study, was naturally releasing seeds in the study area (outside of the experimental chambers) during the course of the experiment. Temperatures in May in North Carolina and June in Massachusetts at the study sites are far from the critical thermal maxima of ant species in the system. For example, the critical thermal maximum for *A. rudis* is 38°C and 40°C for populations at the Harvard Forest and Duke Forest sites, respectively, as opposed to the mean environmental temperatures during the sampling period, which were 20°C at Harvard Forest and 22°C at Duke Forest. The thermal buffer during the time of year when seeds are dispersed may confer some degree of resistance on this mutualism against warming. Our results may suggest that the ant-plant seed dispersal mutualism is protected by the occurrence of most ant-mediated seed dispersal in the spring, when temperatures are still far from the critical thermal maxima of most species. However, phenological shifts in plant reproduction caused by ongoing warming (Price and Waser 1998, Dahlgren et al. 2007, Inouye 2008, Liu et al. 2011, Wolkovich et al. 2012) have the potential to result in seeds appearing before ants become active (Warren et al. 2011). Warren et al. (2011) suggests that while both seed release by plants and onset of foraging in ants seem to be driven by temperature, variability in activation temperatures among ant species may result in situations in which early seeding plant species may become decoupled from their foragers in some areas. Additionally, species may also respond to climate change through adaptation (Kiers et al. 2010, Lavergne et al. 2010), range shifts (Parmesan 1996), or behavioral modification (Barton and Schmitz 2009, Kearney et al. 2009), all of which have the potential to alter interspecific interactions (Walther 2010).

While our results suggest that ant-dispersed plants may not experience reduced seed removal rates as a result of climatic warming, a wide array of other interspecific interactions have been shown to be altered by climate change (Tylianakis et al. 2008, Barton and Schmitz 2009, Harley 2011), including pollinator mutualisms (Memmott et al. 2007, Hoover et al. 2012). Even specific consideration of the impacts of warming on ant-plant seed dispersal mutualisms has suggested that climatic warming will likely decrease seed dispersal by ants (Pelini et al. 2011a, Warren et al. 2011). Inversely, an elevational study of ant-mediated seed dispersal in deciduous forests found declines in seed removal rates in cooler (higher elevation) areas (Zelikova et al. 2008). Not all studies, however, find that climate change alters the nature of interspecific interactions, including mutualisms and facultative interactions (Hegland et al. 2009, Cavieres and Sierra-Almeida 2012), as seems to be the case in this study.

Our finding of a similar lack of response to warming at both study sites differs from many studies, including research done in the same system (Diamond et al. in press), which have documented that organisms at lower latitude sites to often respond more strongly to warming (Deutsch et al. 2008, Tewksbury et al. 2008, Huey et al. 2009, Pelini et al. 2011a, Diamond et al. 2012). These studies often point to proximity of species to critical thermal limits at lower latitude sites as a probable mechanism for stronger responses to warming at lower latitude sites. We again suggest a possible factor conferring resistance on the ant-plant seed dispersal mutualism at both of our study sites may be the spring timing of this mutualism which keeps these ant mutualists far from high temperatures both the northern and southern site. We suggest this as a potential mechanism for the difference between our findings and those of Diamond et al. (in

press), which found several species in the same system to respond to warming at the Duke Forest site.

Despite the apparent resistance of the ant-plant seed dispersal mutualism to experimental warming in this study, there was a significant relationship between the ground-surface temperature at which a species removed seeds and the critical thermal maximum of that species ( $F_{1,4} = 7.35$ ,  $p = 0.05$ ,  $R^2 = 0.65$ ), whereby more seeds were removed under warmer conditions by species with higher  $CT_{max}$  (Figure III.10). The positive relationship between  $CT_{max}$  and seed removal temperature was in agreement with prediction 2 and suggests that while chronic experimental warming may not affect rates of seed dispersal, temperature does relate to rates of seed removal. When incorporating both temperature variability associated with the temperature treatments as well as daily temperature variability, we found species to remove seeds in line with what we would predict based on their thermal tolerance. This finding complements other studies that have shown physiological tolerance to be an important predictor of ant activity (Diamond et al, in press).

One important caveat to our study (and to most studies of ant-seed dispersal mutualisms) is that we do not know the fate of the seeds removed from the experimental seed caches. While we did not detect major differences in seed removal rates across temperature treatments, we also did not examine the ultimate fate of these seeds. It is possible that warming could still alter the dynamics of plant populations by altering rates of germination and seedling survival (De Frenne et al. 2012), even in cases in which seed dispersal remains unaffected as temperatures increases. Additionally, some species, including *C. lineolata*, were observed removing seeds very short distances (only a few centimeters) while other species, such as *C. castaneus*, often carried seeds



several meters. Even slight shifts in dispersal rates among these species may alter plant population dynamics if dispersal distances differ substantially among species.

As organisms respond to climate change, interactions between species may be affected. Based on previous work showing declines in *A. rudis* activity at the southern site with warming and no effect of warming at the northern site, we predicted that the ant-plant seed dispersal mutualisms common throughout deciduous forests of eastern North America might be at risk as the climate warms due to probable declines in the primary dispersal agent (*A. rudis*), particularly near the southern range extent of these forests. However, we found no reduction in rates of seed removal as a result of experimental warming at two distinct geographic locations, nor did we observe differences in species-specific seed removal rates at the Duke Forest site. We suggest that the ant-plant seed dispersal mutualism may be resistant, at least in part, to climatic warming. However, we suggest further research should explore the phenology of mutualisms in areas at risk for extreme climatic changes.

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Table III.5. Test statistics are from linear regressions examining the influence of temperature treatment on the number of seeds removed by each ant species.

<b>Species</b>	<b><i>F</i></b>	<b><i>p</i></b>
<i>Aphaenogaster lamellidens</i>	2.58	0.58
<i>Aphaenogaster rudis</i>	2.45	0.15
<i>Camponotus castaneus</i>	0.10	0.75
<i>Camponotus pennsylvanicus</i>	0.24	0.64
<i>Crematogaster lineolata</i>	4.14	0.07
<i>Formica pallidefulva</i>	0.34	0.58
<i>Formica subsericea</i>	0.02	0.90



Table III.6. Percentage of seeds removed by each species overall, during the day, and the night.

Chi-square and associate *p* values indicate differences between seed removal day and night.

<b>Species</b>	<b>Overall percent</b>	<b>Day percent</b>	<b>Night percent</b>
<i>Aphaenogaster lamellidens</i>	8.5	17.2	0.0
<i>Aphaenogaster rudis</i>	45.5	48.3	42.7
<i>Camponotus castaneus</i>	26.7	0.0	52.8
<i>Camponotus pennsylvanicus</i>	2.8	2.3	3.4
<i>Crematogaster lineolata</i>	6.8	12.6	1.1
<i>Formica pallidefulva</i>	2.3	4.6	0.0
<i>Formica subsericea</i>	7.4	14.9	0.0



Figure III.8. Picture of Duke Forest warming chamber.

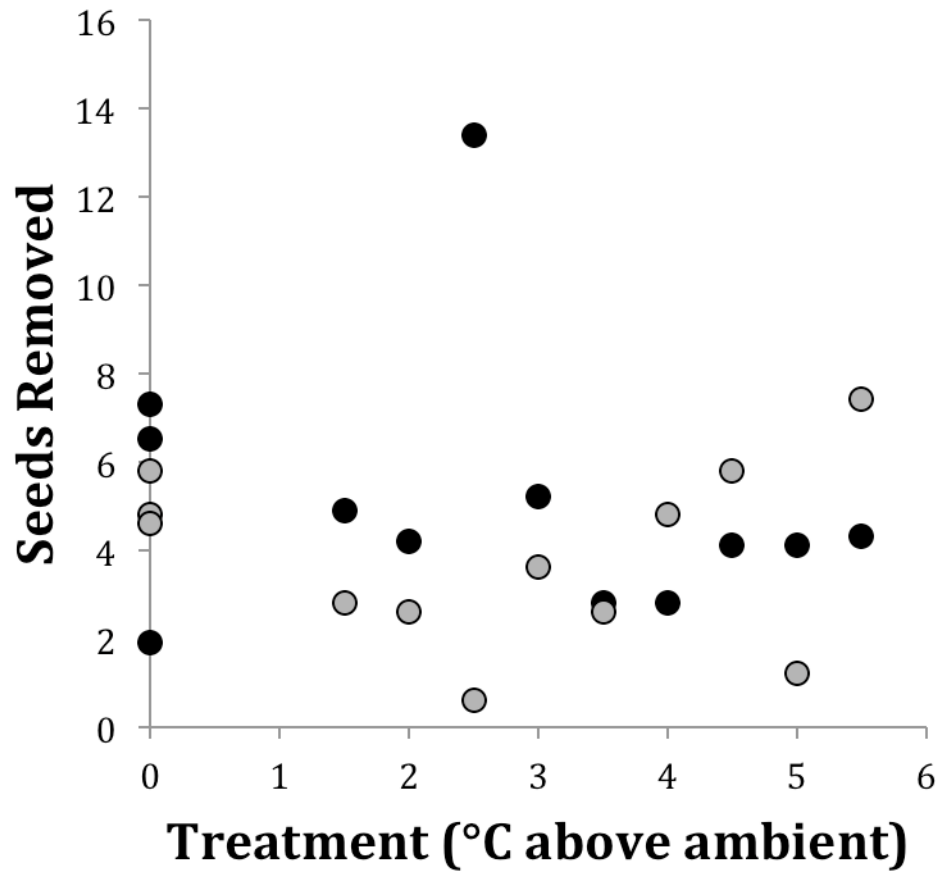


Figure III.9. Number of seeds removed in the course of an hour as a function of temperature treatment. Black dots represent Duke Forest and gray dots represent Harvard Forest.

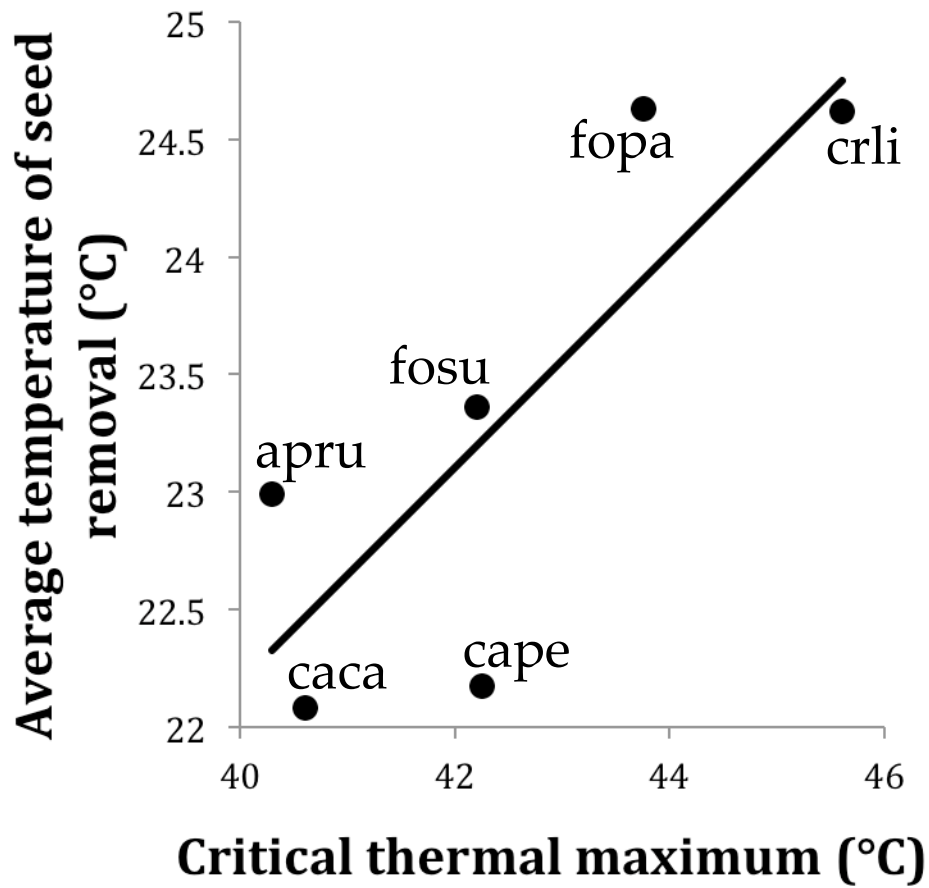


Figure III.10. Number of seeds removed in the course of an hour as a function of temperature treatment. Black dots represent Duke Forest and gray dots represent Harvard Forest.

## CONCLUSIONS

My dissertation examined the structure of ant communities and how climatic warming may alter these communities and their function. I found segregation of time to be an important mechanism in promoting the coexistence of ants within an eastern deciduous forest, while temperature and space were not segregated, nor was there evidence for either the dominance-discovery or dominance-thermal tolerance tradeoff. This is one of the first studies to simultaneously examine the importance of a suite of potential coexistence mechanisms within a study system. It also introduces a new method for examining dominance hierarchies in ant communities, providing confidence intervals around dominance estimates.

Further, I found evidence that climate change may alter ant communities. Experimental warming influenced levels of ant foraging activity, shifting overall rates of foraging activity as well as species-specific rates. Species with higher critical thermal maxima foraged more heavily at hotter temperatures than species with lower critical thermal maxima. Additionally, ants at the two study sites exhibited differing patterns in response to warming. Only the southern site showed any shifts in ant activity under warmed conditions while there was no response at the northern study site. However, this shift in ant activity did not translate into shifts in rates of ant-mediated seed removal. So, while experimental warming altered rates of ant activity, warming did not alter rates of seed removal by ants in either study system. This work has important implications for the future by potentially enhancing our ability to make predictions regarding the responses of individual species to climatic warming, but also suggests limitations in predicting the indirect effects of warming on communities without the use of manipulative experiments.

While the research outlined in chapter 2 of my dissertation provides some insights into the mechanisms that may promote coexistence among ant species, there is still much to be learned in this field. One issue that arises is the actual importance of behavioral dominance in the structuring of ant communities. Behavioral dominance is commonly considered an important trait in tradeoffs promoting coexistence in ant communities. However, the actual link between aggression and food acquisition is not well understood. Further research into the actual importance of behavioral aggression in multiple study systems would be highly informative in the field.

When considering the effects of climatic warming on ant communities, the extent to which competitive interactions between ant species drive changes in community composition and foraging activity is not clear. Controlled manipulations in the lab and/or field would be useful in determining the extent to which shifts in foraging activity and relative abundance are caused by temperature alone, as opposed to interactions between temperature and interspecific competition.

Additionally, while we found no relationship between experimental warming and rates of seed removal by ants, it is still possible that climatic warming may disrupt this mutualism through a variety of mechanisms including potential phenological mismatches between ants and plants or altered seed fate resulting from variation in the handling of seeds by ants. For example, warming could potentially change the distance seeds are carried or the destination to which ants move them.

While there is still much research to be done to better understand the forces structuring ant communities as well as the potential for climatic warming to alter these communities and the

processes they mediate, my dissertation begins to address these issues. The results of is research will aide in our understanding of how ant communities are assembled and how these communities, as well as associated organisms, may be affected by climatic warming in the coming century.

## **APPENDICES**



## Appendix I.1

The C-score for each species-pair calculates the respective overlap of the two species at baits as  $(R_i - S)(R_j - S)$ , where  $R_i$  is the number of occurrences for species  $i$ ,  $R_j$  is the number of occurrences for species  $j$ , and  $S$  is the number of baits at which they both occur. The observed C-score for the entire community is the average of all of the pairwise comparisons. We compared the observed C-score to the C-scores generated from 5,000 null communities using EcoSim Version 7.72 (Gotelli and Entsminger 2010). We used the fixed-fixed null model to construct the null communities, which maintains row and column totals (i.e. maintaining the number of occurrences of each species as well as the number of species occurring in each site) to create null communities. An observed C-score that is significantly greater than the C-scores from the null communities indicates that species within the community segregate space more than would be expected in the absence of the proposed mechanism, while a C-score that is substantially lower than the simulated C-score indicates that species within the community tend to be aggregated more than would be expected by chance alone.

## **Appendix I.2**

The Czechanowski index was calculated for all pairwise groups and then averaged for the community. We compared the observed averaged overlap indices to the overlap indices generated from 1000 randomizations. Niche breadth for each species was maintained. We then compared the observed Czechanowski indices to indices resulting from the randomized data (see (Albrecht and Gotelli 2001) for more details). Observed overlap indices that are significantly greater than the randomizations indicate a greater degree of niche overlap than would be expected by chance while index overlap indices that are significantly lower than the randomized values indicate a lower degree of niche overlap than expected.

Table I.7. Dominance indices based on the Colley ranking method and proportion of aggressive encounters won.

Ranked by proportion wins:

Species	Dominance
<i>Camponotus pennsylvanicus</i> (De Geer)	0.96
<i>Crematogaster lineolata</i> (Say)	0.94
<i>Prenolepis imparis</i> Emery	0.93
<i>Camponotus americanus</i> Mayr	0.89
<i>Camponotus castaneus</i> (Latreille)	0.82
<i>Tapinoma sessile</i> (Say)	0.67
<i>Formica pallidefulva</i> Latreille	0.53
<i>Formica subsericea</i> Say	0.5
<i>Aphaenogaster lamellidens</i> Mayr	0.2
<i>Aphaenogaster rudis</i> Enzmann	0.17
<i>Nylanderia faisonensis</i> (Forel)	0.12
<i>Temnothorax curvispinosus</i> Mayr	0.03

Supplemental Figure I.11. Median discovery times and 50<sup>th</sup> quartile of data for the six most common ant species observed in discovery trials. Discovery times only reflect events for which the species was the first to discover the bait.

Supplemental Figure I.12. Maximum, minimum, and mean ground temperatures at which ants were observed foraging during the day, along with the range of ground temperatures observed.

Supplemental Figure I.13. Co-occurrence patterns for (a) all ants, (b) dominant ants, and (c) subdominant ants. Bars show the distribution of C-scores in the null communities and arrows indicate where the observed C-score falls.

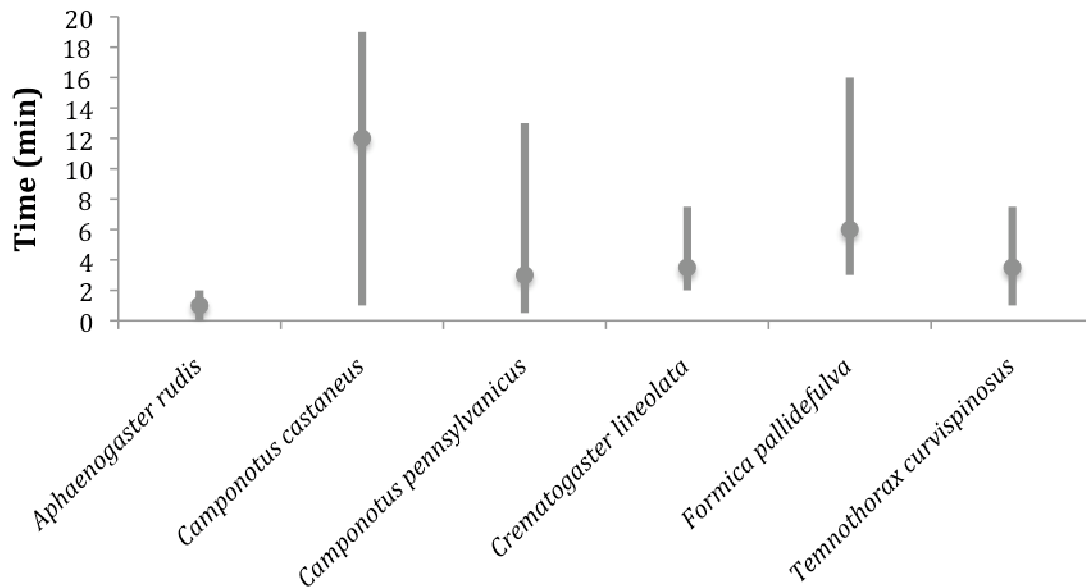


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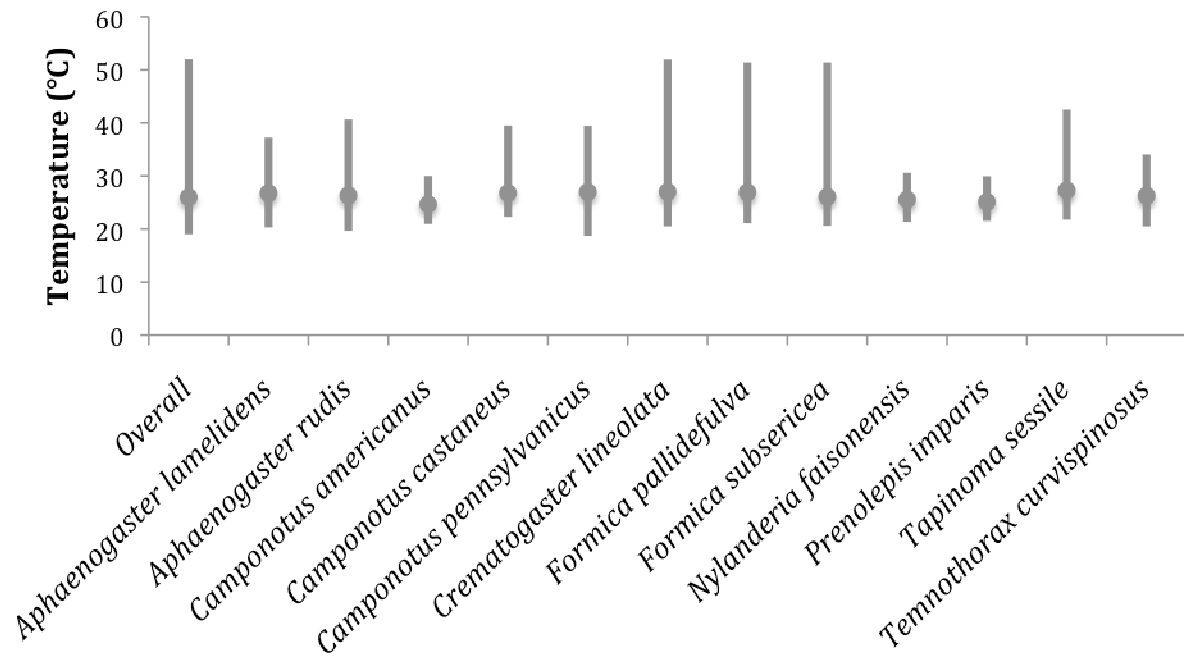
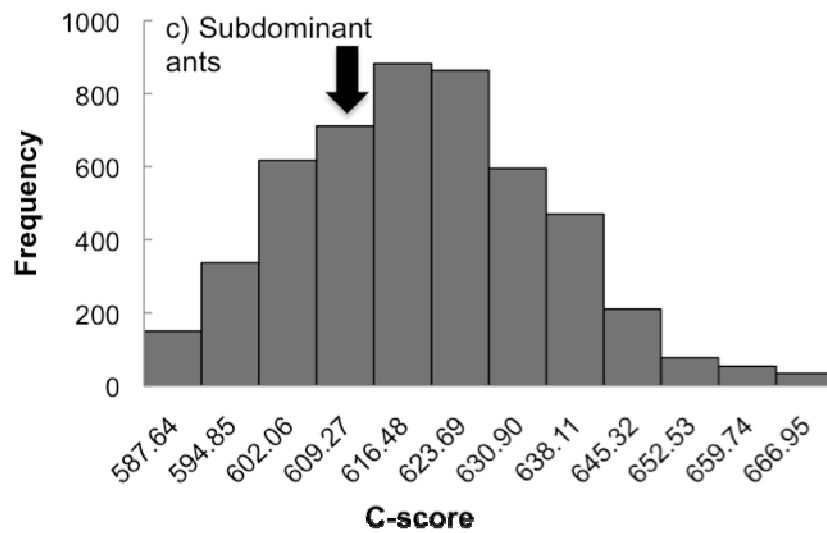
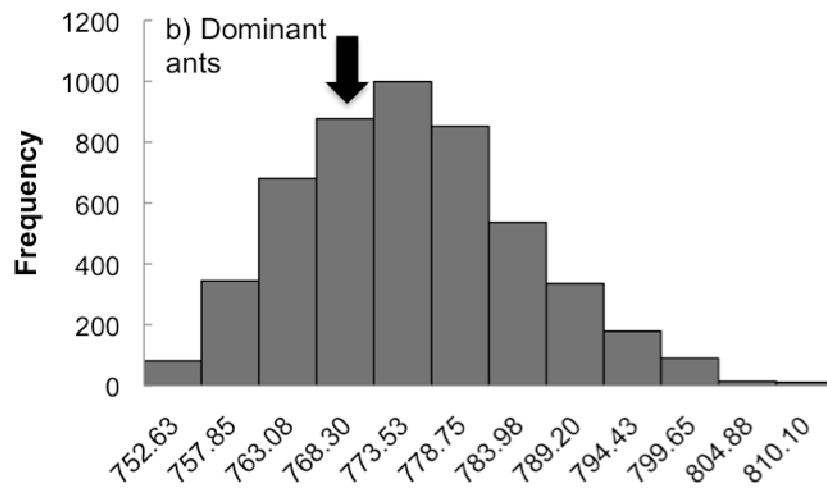
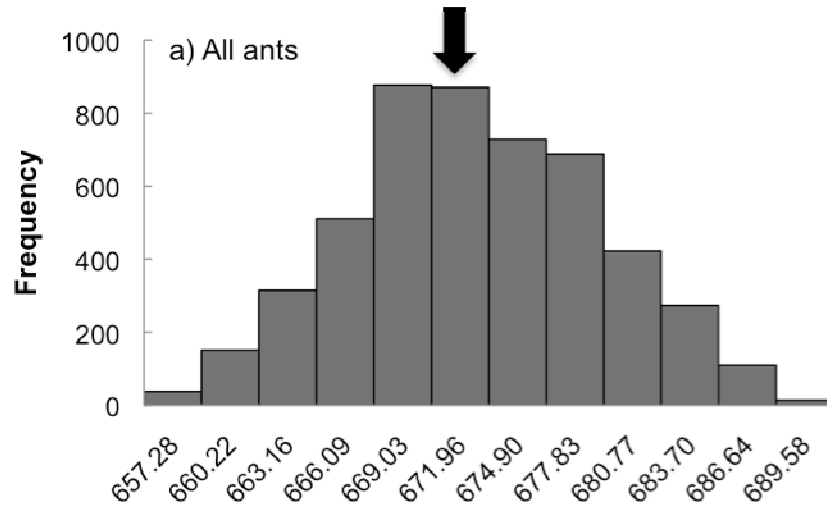


Figure I.12. Maximum, minimum, and mean ground temperatures at which ants were observed foraging during the day, along with the range of ground temperatures observed.

Figure I.13. Co-occurrence patterns for (a) all ants, (b) dominant ants, and (c) subdominant ants. Bars show the distribution C-scores in the null communities and arrows indicate where the observed C-score falls.





## **VITA**

Katharine Stuble was born in southern Maryland. She graduated with a B.A. in biology from St. Mary's College of Maryland in 2004. In 2008 she graduated from the University of Georgia with a M.S. in ecology, having completed a thesis on the ecological impacts of red imported fire ants on longleaf pine savannas. In 2008, Katharine began work toward a Ph.D. in the Department of Ecology and Evolutionary Biology at the University of Tennessee.