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Linking physiology and biogeography: Disentangling the constraints on the distributions of ant species

Lacy Danikas Chick
University of Tennessee - Knoxville, ldanikas@vols.utk.edu

To the Graduate Council:

I am submitting herewith a dissertation written by Lacy Danikas Chick entitled "Linking physiology and biogeography: Disentangling the constraints on the distributions of ant species." 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:

Daniel Simberloff, Aimee Classen, Ernest Bernard, Alison Boyer

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Linking physiology and biogeography: Disentangling the constraints on the distributions of ant species

A Dissertation Presented for the
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To Sullivan – Never give up

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ABSTRACT

Understanding the factors that limit the distribution of species is at the core of ecological and biogeographical research, and is critical if we are to predict the responses of key ecosystem components to ongoing climatic changes. My doctoral research seeks to provide an understanding of how thermal physiology influences species' distributions and better define the mechanisms underlying geographic variation in biodiversity. By using natural temperature gradients (both elevational and latitudinal) and coupling controlled laboratory experiments with field observations and null modeling approaches, I was able to document the role of inter-specific variation in thermal physiology and, more interesting, inter-population variation in thermal physiology, in shaping the distribution of diversity on a warming planet. I determined that species' density and distributions are shaped by both biotic and abiotic factors, but that the influence of these factors is geographically-dependent. I further examined the role of temperature by determining how different rates of warming affect thermal physiology and might provide insight into separate aspects of an organism's life history and its accompanying coping mechanisms. Finally, I used a common garden experiment and phylogenetic analyses to determine to what extent ecological and evolutionary forces play a role in shaping the thermal niche. I found patterns suggestive of local adaptation and no evidence for lab acclimation, suggesting that some species may have limited acclimation ability and therefore will be more susceptible to climate warming. This dissertation suggests that variation in thermal physiology within and among species is important in understanding the factors that shape diversity and how species will be distributed now, and in the future.

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INTRODUCTION

Global climate change has altered communities through range shifts of particular species (Root *et al.*, 2003; Chen *et al.*, 2011; Crimmins *et al.*, 2011), phenological changes (Parmesan and Yohe, 2003; Warren *et al.* 2011), and local extinctions (Sinervo *et al.*, 2010). Few studies, however, have examined the physiological mechanisms underlying these changes or the impact they might have on community structure (but see Buckley *et al.* 2010). For ectotherms, temperature is one of the most important abiotic factors affecting the distribution and abundance of species (Andrewartha and Birch, 1954; Hawkins *et al.,* 2007; Currie *et al.*, 2004) and spatial variation in climate can increase the potential for high inter-population variability across the range of a species (Mizera and Meszéna, 2003).

Thermal environments often covary with latitude and elevation, frequently creating extensive thermal gradients. However, climatic warming will likely not be consistent along contemporary environmental gradients. Temperature has been considered a key factor in limiting range shifts of organisms because of regional adaption to thermal regimes. Ectotherms living in warmer climates experience temperatures closer to their upper thermal limits and therefore are considered to be more vulnerable to rapid warming (Deutsch *et al.*, 2008; Kingsolver *et al.*, 2013). Thus, if warming is not consistent along environmental gradients, climate change could result in sub-optimal environmental temperatures for longer periods at extreme elevations and latitudes, thereby influencing physiological processes and behavioral interactions for a suite of organisms (van Damme et al., 1989; Huey and Kingsolver, 1993).

Variation in the physiology and behavior of key species along environmental gradients can have cascading effects on community membership and interspecific interactions. Examining trait variation and local adaptation is especially important for understanding how environmental change will affect communities. By assuming all populations of a species respond identically to climatic variables, most models and previous studies have disregarded a fundamental premise of evolution by natural selection — variation. It is expected that the magnitude of warming will be heavily dependent on geographic location (IPCC, 2013). Still, few studies relate the physiological factors mediating organismal performance to range size and distribution with respect to climate change and population dynamics (but see, Addo-Bediako *et al.*, 2000; Buckley *et al.*, 2010; Sunday *et al.*, 2011). As communities are altered by global change, variation among populations will likely lead to novel communities in some areas, while other areas might see reductions in species richness due to range shifts and/or local extinctions (biotic attrition) (Colwell *et al.*, 2008). Documenting the mechanisms that link physiological traits to geographic distributions will likely aid in predicting potential changes in community structure by taking into account organismal performance and future environmental factors.

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CHAPTER I
THE GEOGRAPHY OF ECOLOGICAL PROCESSES: THE INTERPLAY BETWEEN BIOTIC AND ABIOTIC FACTORS ON THE DISTRIBUTION AND DENSITY OF SPECIES

This chapter is currently in review at *Journal of Animal Ecology*

Co-authored by Jean-Philippe Lessard, Robert R. Dunn, and Nathan J. Sanders

Author contributions: All authors made substantial contributions to the concept and experimental design of this study and contributed to data analysis and writing of this manuscript. JPL and NJS collected abundance, density, and dominance data and LDC carried out field collections and all thermal physiological work. NJS and RRD conceived and coordinated the study. All authors gave final approval for publication and inclusion in this dissertation.

ABSTRACT

Both biotic and abiotic factors shape the distribution of life on Earth, but their relative influences likely vary spatially. Here, we couple field based observations, null model analyses, and laboratory-measured physiological thermal limits to examine the interplay of climate and species interactions in structuring ant communities along an extensive abiotic gradient. We found that both temperature and species interactions shape the abundance, distributions, and density of ant species. However, the strength of the influences of the biotic and abiotic factors was context-dependent. Environmental conditions tended to be more important in colder, more stressful environments, where physiology was the most important constraint on the distribution and density of ant species. Conversely, the influence of species interactions was highest in warmer, more benign conditions. Such a pattern, first suggested by Fischer in 1960, but then largely ignored empirically, suggests that the response of species to climate change, whether historic or future, is likely to be context-dependent and more specifically, geographically dependent. In temperate regions, where most experimental studies of climate change are done, responses may be far easier to predict than in tropical regions where they will depend not only on the physiology of organisms but also on their interactions.

INTRODUCTION

One of the most striking patterns in nature is that the number of species varies, often systematically, along environmental gradients. Explaining this pattern has attracted the attention of ecologists and biogeographers for decades (MacArthur *et al.*, 1972), if not longer (Humboldt, 1849) and has inspired empirical studies in fields ranging from physiological ecology to macroecology and global change biology (Diez *et al.*, 2012). But why does the number of species that coexist in a particular assemblage vary? One possibility is that, broadly speaking, species differ in how they respond to biotic and abiotic factors along environmental gradients, and these differences among species, in turn, influence abundance, distribution, community composition, and broad-scale patterns of diversity. For instance, temperature tends to decrease systematically with elevation and latitude (Fridley, 2009; McCain & Colwell, 2011) and as a result, the abiotic environment at high-elevation and high-latitude sites might be more physiologically stressful for potential colonizers than at low-elevation and low-latitude sites. In such a model, temperature acts as a filter, permitting the occurrence of only those species with traits that allow them to persist at low temperatures (Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011).

Of course, multiple factors can and do simultaneously operate to shape communities, and different factors might be more important in different locations (Sundqvist *et al.*, 2013) Wallace (1878), Dobzhansky (1950), and Fischer (1960) all suggested that negative interspecific interactions (competition, predation, parasitism) might be more intense or important in benign, stable environments. Indeed, a growing number of investigators have begun to explore the geography of biotic interactions (Schemske *et al.*, 2009) with recent studies suggesting that negative interactions might limit the distributions of species and pose a cap to the

number of species that can coexist in benign environments, (Jankowski *et al.*, 2010; Kozak & Wiens, 2012). Two recent studies along elevational gradients hint at such a scenario: in hummingbird assemblages in the Andes (Graham *et al.*, 2009) and in ant assemblages in the U.S. and Europe (Machac *et al.*, 2011) there is some evidence that interspecific interactions shape community membership at low elevations, but that more stressful environmental conditions (e.g., cold temperatures) shape communities at high elevations. Such studies are important because they suggest a mechanism, but, they do so based on community phylogenetic approaches, which rely on numerous underlying assumptions and can give misleading answers about the processes that actually structure communities (Losos, 2008; HilleRisLambers *et al.*, 2012).

More compelling evidence for geographic variation in the relative influence of climate and biotic interactions on the species in assemblages might come from field-based measurements of physiological tolerances (e.g., (Helmuth *et al.*, 2002; Sinclair *et al.*, 2006; Buckley *et al.*, 2008) and/or detailed studies of the outcomes of interactions among species, i.e., actual measurements of individual-level functional traits and observations of interactions in the field (Albrecht & Gotelli, 2001; Parr, 2008; Stuble *et al.*, 2013; Violle *et al.*, 2012). Such studies, however, are rare because they are time consuming and impossible for many of the groups of organisms on which studies of geographic gradients tend to focus. Additionally, many traits that are often measured do not directly relate to tolerance of the abiotic environment.

Like other ectotherms, ants exhibit thermal sensitivity, and species differ in their thermal tolerances (i.e., the ability to tolerate either extreme temperatures or a broad range of temperatures; (Cerdá *et al.*, 1997; Diamond *et al.*, 2012; Kaspari *et al.*, 2015). Thermal tolerance in ants may be related to total abundance and range size (Geraghty *et al.*, 2007; Warren & Chick, 2013) foraging activity (Cerdá

et al., 1998; Lessard *et al.*, 2009; Stuble *et al.*, 2013) and broad-scale patterns of diversity (Kaspari, 2000; Sanders *et al.*, 2007). If a species occurs at all locations with suitable environmental conditions, then the environment alone would be the sole driver of its distribution. However, if the observed range of a species is smaller than its expected range based on environmental tolerance alone, then some other factor, such as competitive interactions or dispersal limitations, acts to shape the distribution of species among local communities along the gradient (Guisan & Rahbek, 2011; Fordham *et al.*, 2013). If the same suite of factors affects the distribution of many species, then such factors are expected to also influence the distribution of diversity, and diversity is simply a collective property.

Competitive interactions are widely thought to influence the structure and dynamics of some local assemblages, and might shape broad-scale patterns in the distribution of species as well. Competition likely structures local ant assemblages (Cerdá *et al.*, 2013), yet its effects are mediated by temperature altering interactions between dominant and subordinate species (Bestelmeyer, 2000; Cerdá *et al.*, 1997; Cerdá & Retana, 1998; Lessard *et al.*, 2009) and the activities of particular species (Lessard *et al.*, 2009; Stuble *et al.*, 2013). Here, we examine non-random co-occurrence patterns among ant species along the environmental gradient to assess whether species occur less than expected by chance as would be expected if competition structures communities (Gotelli & McCabe, 2002). Additionally, we examine how the abundance of competitively dominant species affects species density in a local assemblage. Species density (the observed number of species in a defined area) may be negatively related to the abundance of dominant species, such that as the abundance of dominant species increases, subordinate species are competitively excluded and species density declines. Such a pattern is common, at least when ant assemblages are invaded by competitively dominant non-native species (Holway *et al.*, 2002). Alternatively, the relationship between the abundance of dominant species and

density in the rest of the assemblage may be unimodal (Andersen, 1992; Parr *et al.*, 2005; Parr, 2008). Such a pattern might occur in response to environmentally stressful conditions, which limit both dominant and subordinate species. But as conditions improve, the abundance of dominant species and density of subordinate species increases until the abundance of the dominant species becomes so high that the dominant species begin to limit subordinate species (Andersen, 1992; Parr *et al.*, 2005; Parr, 2008).

In this study, we ask a series of inter-related questions about the factors that govern the distribution, abundance, and density of ant species along an extensive and well-studied elevational gradient in Great Smoky Mountains National Park, USA. In particular, we ask (1) Are abundance and species density correlated with environmental temperature? (2) Does thermal tolerance predict elevational range size and species density as would be the case if temperature were the sole driver of species distributions? (3) Do species co-occur less among assemblages than would be expected if temperature alone limits membership? (4) Does competition by dominant species affect species density among assemblages? Based on the suspicions of early biogeographic pioneers (e.g., (Fischer, 1960; Wallace, 1878; Dobzhansky, 1950), we predicted that physiological constraints would limit community membership at high-elevation sites, filtering species that have the physiological capacity to withstand more extreme temperatures, but that interspecific interactions shape assemblages at lower elevation sites that are more environmentally benign. We further predicted that thermal tolerance would be the best predictor of the occurrence and number of species in high elevation communities where environmental filtering predominates, but not at low elevation where biotic interactions are most frequent and intense.

METHODS

Sampling

We did this work in Great Smoky Mountains National Park at sites that were situated in mixed hardwood forests and were located in areas away from roads, heavily visited trails, or other recent human disturbances. We systematically sampled 29 sites (from 379 to 1828m) in June-August 2004 – 2007. These sites had a temperature range of -8.0 – 29.7°C (mean annual temperatures ranged from 7.7 – 13.3°C) and ranged from 1308 – 1928 mm in annual precipitation. We used Winkler samplers to extract ants from the leaf litter in 16 1-m^2 quadrats at each site in a haphazardly placed 50 × 50 m quadrat. At each site, species density is the observed number of species collected in the 50 × 50 m quadrat, and abundance is the number of 1-m^2 quadrats in which any species was detected. This estimate of abundance (which is actually "occurrence" (Kaspari *et al.*, 2000; Longino *et al.*, 2002; Sundqvist *et al.*, 2013; Gotelli & Colwell, 2010; Gotelli *et al.*, 2011)) is preferable to a count of worker number because ants are social, and because counts of colonies is challenging when species have multiple nests per colony and occur in the leaf litter. We differentiate "abundance" from "occurrence" because our measure of abundance combines all species whereas "occurrence" implies the presence of only a single species. At eight of the sites, we also collected ants using an array of 10 pitfall traps over 2 years (Lessard *et al.*, 2007). The number of species collected by pitfall traps did not differ from the number collected by the Winkler samplers (paired $t = 1.88$, $n = 8$, $P = 0.11$). Similarly, the fauna sampled by the pitfall traps was similar to the fauna sampled by the Winkler samplers (Lessard *et al.*, 2007). At most of the sites, an asymptotic species richness estimator (Chao2 in this case) plateaued, suggesting that sampling within sites approached completeness (Sanders *et al.*, 2007). Moreover, a Chao2 estimate of richness among all sites suggests, at least using these sampling techniques at similar sites, that there would be

approximately 45 species in total, and we captured 38 species in our systematic sampling. Therefore, these communities are adequately sampled.

In July 2011 and 2012, we visited 31 sites (from 375-1825m; 17 of which were in the previously sampled sites in 2004-2007) in order to collect live individual ants for physiological tolerance estimates. At each of these 31 sites, we used the same Winkler extraction methods as in the previous sampling to extract ants from the leaf litter. However, we collected litter from only 10 1-m^2 quadrats per site instead of 16, and we extracted live ants from the leaf litter by sifting through the litter in the field rather than returning them to the lab to use Winkler extractors, as is typically the case with Winkler sampling methods. We made these modifications because we were not aiming to sample the entire community and because we needed live specimens. Finally, we also baited for ants by placing laminated index cards stocked with ~5 g of tuna in oil and hand collected individuals at the site. For any species we detected either at the bait, the Winkler extraction method, or in general hand collecting, we obtained 10 live individuals and returned them to the lab (~1-2 hours from the field site) to estimate thermal tolerance.

Estimating thermal tolerance

We used critical thermal minima (CT_{min}) and maxima (CT_{max}) to examine the physiological constraints imposed on species across the environmental gradient. For each species collected at each site, heat and cold tolerance experiments were performed on 5 individuals for CT_{min} and 5 individuals for CT_{max}, which were estimated by documenting the temperature at which individuals lost the ability of righting response. Loss of righting response is measured as the point in which an organism is flipped on its dorsum and can no longer independently right itself. This measure is considered an ecologically relevant endpoint for physiological tolerance (Lutterschmidt & Hutchison, 1997) because as an organism becomes

incapacitated, it can no longer forage or escape predation. We used methods described in Warren & Chick (2013) to estimate thermal tolerance for each species at each of the 31 sampled sites. Individuals were transferred to 16mm glass test tubes, plugged with cotton to reduce thermal refuges, and were placed in an Ac-150-A40 refrigerated water bath (NesLab, ThermoScientific). Water bath temperatures were raised or lowered at a rate of $1°min^{-1}$ until thermal tolerance was reached. We characterized thermal tolerance as the highest and lowest temperatures at which an individual could no longer retain locomotor ability, respectively. One vial contained only a copper-constantan Type-T thermocouple (Model HH200A, Omega, Connecticut, USA) and was used to monitor temperature inside the tubes and to ensure accurate readings. We performed all tolerance tests within 5 hours of field collection to reduce potential acclimation to the lab thermal environment; however, a subsequent common garden experiment indicated no effects of acclimation on thermal limits. A mean temperature of the loss of righting response served as the index for thermal tolerance for each species at each site. We preserved all ants individually in 2.0-mL vials containing 95% ethanol, and placed them in NJS's private collection at the University of Tennessee.

Are abundance and species density correlated with environmental temperature?

We extrapolated current (~1950-2000) mean temperatures for each site from the WorldClim database (Hijmans *et al.*, 2005) at a resolution of 30 arc-seconds. Previous work in this region (Fridley, 2009) modeled climate based on empirical data collected from a 120-sensor temperature logger network. While data from the 120-sensor network are more fine-scaled, they were not used in this study because they were collected for a shorter time period (2005-2006) and because temperature measured in the data loggers is correlated with elevation in much the same way as WorldClim data. Similarly, data from weather stations arrayed

in the region indicate that temperature declines in a manner comparable to the model used by WorldClim. For these reasons, we used the WorldClim dataset here so that our findings may be more comparable to studies along other gradients where fine-scale resolution may not exist.

We plotted total abundance (the total number of 1-m^2 quadrats in which a species was detected, combined for all species) and species density (the number of species in the 50 × 50 m quadrat) against mean annual temperature (MAT; we note that MAT was strongly correlated with both January minimum and July maximum). We used least squares regressions to examine the relationship between temperature and total abundance as well as the relationship between temperature and species density. If temperature is an important determinant of species density and abundance, and colder temperatures filter species from the regional species pool, we would expect to find a linear relationship in which both species density and abundance declined with decreasing temperature.

Does thermal tolerance predict elevational range size and species density as would be the case if temperature were the sole driver of species occurrence?

To test whether physiological tolerance of environmental temperature influences spatial variation in species density, we examined the relationship between the thermal ranges of species (i.e., $CT_{max} – CT_{min}$) and the environmental conditions across the gradient. We first asked whether species with broader thermal tolerances had broader elevational ranges and higher elevational midpoints. For each species we combined the sampling data and plotted the highest elevation at which it was collected minus the lowest elevation at which it was collected and determined the elevational range of each species. To calculate elevational midpoints, we calculated the mean of the highest elevation and lowest elevation at which each species was collected (Rohde *et al.*, 1993). We then related these

values to the thermal range of each species. We predicted that if temperature were an important determinant of the range sizes of species, then species at higher elevations that are able to withstand colder temperatures (i.e. high-elevation species) would have broader thermal ranges than species at lower elevations that may be confined by their physiological temperature tolerances. Species with broader thermal ranges typically have broader geographic ranges and thereby also have higher elevational midpoints (Sanders, 2002).

Many species likely overlap in the range of temperatures at which they can occur based on their physiological thermal ranges. Yet if species do not occupy the same environmental conditions as would be predicted by their thermal tolerances alone, then some other factor accounts for at least some of the variation in species density and occurrence. To determine whether thermal tolerance influenced species density, we asked whether the species occurring in a particular community were simply the collection of those species whose thermal tolerances overlapped the annual range of temperatures of that particular place. To do this, we extracted the annual range of temperatures (maximum temperature of the warmest month - minimum temperature of the coldest month) for each of the 27 of the 29 sites for which we had estimates of species density and calculated the mean maximum and minimum thermal limits of each of the 18 species across the 27 sites for which we had thermal tolerance data (two sites were omitted because species found at these sites did not have thermal tolerance data and therefore we could not estimate expected densities). We then calculated the extent of overlap between physiological ranges of the ants and environmental temperatures of the sites (henceforth, thermal overlap). For any given species × site combination, this is simply the range of shared temperatures for both the species and the site. We then used these values to estimate a probability of occurrence for each species at each site using logistic regression models.

In the logistic regression models, the probability of occurrence of one species was determined based on its thermal overlap, as well as the thermal overlaps and recorded presences of the other 17 species in the regional species pool. This approach allowed us to determine a probability of occurrence for each species at each site based on overlapping physiological and environmental conditions (thereby incorporating variation in physiological thermal ranges and environmental thermal ranges between sites), as well as actual occurrences of other species (thereby incorporating the possibility for species co-occurrences). So as not to bias the models, we did not include presence data for the focal species when estimating the probability of occurrence of that species, as including the actual occurrence of a species would inherently increase its probability of occurring in a given area.

Finally, to estimate expected species density based on thermal overlap alone, we simply summed the independent probabilities of occurrence for each species at each site. This expected species density would then be the number of species that could occur at a particular place along the gradient if temperature and temperature alone limited community membership. We then plotted observed species density against the expected species density based on thermal limits alone. If the slope of the line of expected species density plotted against observed species equals 1, then temperature would be the sole predictor of species density. Both presences and absences of species are evident in the site × species matrix. One possibility is that the absences were not true absences. So, as a test whether the potential pseudo-absences in the site × species matrix could influence the result, we filled in the matrix so that all sites between the highest and lowest elevation at which a species was recorded were counted as presences. We then compared the expected species density if each species

occurred at every site within its range to the predicted range based on thermal tolerance alone.

Do species co-occur less among assemblages than would be expected if temperature alone limits membership?

We used null model analyses to ask whether species co-occur non-randomly (some species pair combinations being less frequent than expected by chance alone) among sites, as would be predicted if competitive interactions influenced the distribution of ants. In particular, we used the C-score of Stone and Roberts (1990) to quantify co-occurrence patterns. The C-score quantifies the number of "checkerboard units" for each species pair, where a checkerboard unit is a 2 × 2 submatrix of the form 01 10 or 10 01. For each species pair, the number of checkerboard units is $(R_i - S)(R_j - S)$, where R_i is the number of occurrences (equal to the row total) for species i, R_j is the number of occurrences for species j, and S is the number of sample plots in which both species occur. The C-score is the average number of checkerboard units for each unique species pair. If this index is unusually large compared with a null distribution, there is less pairwise species co-occurrence (segregation) than expected by chance. If the index is unusually small, there is more species co-occurrence (aggregation) than expected. We compared the observed C-scores to those generated from 5000 randomly constructed assemblages (using null models in EcoSim version 7.72, (Gotelli & Entsminger, 2005)). C-scores that are not significantly larger than expected by chance indicate random species distributions among sites, and C-scores that are smaller than expected by chance indicate species aggregation. We used fixed-fixed null model (Gotelli, 2000) for which both row totals and column totals are fixed within sites and among species, which maintains differences in species density among sites and total occurrences among species. Gotelli (2000) suggests that SIM9 is appropriate for analyzing co-occurrence patterns of species from "island lists" and has a low probability of Type I errors.

We conducted this analysis for all 29 sites to determine a general pattern, and then for the 12 communities at high (>1000m) and 17 communities at low (<1000m) elevations separately to examine whether the signature of competition varied along the environmental gradient.

Does competition by dominant species affect species density among assemblages?

The relationship between the abundance of dominant species and species density in the rest of the assemblage is predicted from competition theory to be either linearly decreasing or unimodal. There are many ways to quantify dominance in ant and other assemblages (e.g., (Stuble *et al.*, 2013); see (Cerdá *et al.*, 2013) for a review). Here, we used data from observations at bait stations to identify dominant species based on the outcomes of direct interference interactions and the ability to monopolize bait stations.

We combined data from two separate studies conducted in this system to maximize the number of observations upon which we based our rankings. In a first study, we randomly selected a subsample of 15 sites from the original 29 sites surveyed in 2004-2007. At each of 15 selected sites, we haphazardly positioned two white laminated index cards stocked with honey water and two more cards stocked with tuna baits on the ground. Tuna baits consisted of a teaspoon of canned tuna whereas honey baits were cotton balls dipped into a 5% honey solution. Every 15 minutes for an hour, we visited each bait station and recorded the outcome of behavioral interactions. We repeated this procedure 4 times at each site from June to September 2007. In a second study, carried out in June-July 2008 and 2009, we randomly selected 10 sites in a lowland mixed hardwood forests. At each site we positioned 12 bait stations, 5-m apart, in a 15m × 20m grid. Each bait station consisted of a teaspoon of cat food positioned at the center of a white laminated index card. We visited bait stations and

recorded the outcome of behavioral interactions every 15 minutes for a period of three hours. For this study, we visited each site only once.

To determine which species were competitively dominant, we calculated the proportion of interactions won by each species at baits based on a total of 1920 observations at bait stations. For each observation, we recorded the outcome of the first inter-specific interaction observed. A “win” consisted of a species attacking another one and leading to the submissive species leaving the bait station (which we then counted as a loss for that species). We then used the bias-free Colley ranking method (Colley, 2002) to rank species from most dominant to most submissive (Feener *et al.*, 2008; Lebrun & Feener, Jr. , 2007; Stuble *et al.*, 2013). The Colley method estimates the dominance hierarchy based on (i) the proportion of “win” interactions, and (ii) the relative strength of the opponents in inter-specific interactions. Thus, winning an interaction against a dominant species is worth more than winning against a submissive species. The Colley method was designed to rank American college football teams; it does not require that every species interact with one another to obtain an accurate ranking. Therefore, the Colley method is more robust than previously used methods (Andersen, 1992; Sanders & Gordon, 2003). We then ranked each species based on the Colley ranking and on the ability of species to monopolize baits. Four species were identified as behaviorally dominant species: *Formica subsericea, Prenolepis imparis, Lasius alienus*, and two species that were virtually indistinguishable in the field - *Camponotus pennsylvanicus* and *C. chromaiodes.*

Finally, we examined the relationship between the relative abundance of dominant species and species density in the rest of the assemblage by plotting species density of the non-dominant species against the relative abundance of dominant species, where relative abundance was calculated by dividing the total

number of occurrences of the dominant species in the 1-m^2 quadrats by the total number of occurrences for all species at the site. If more than one dominant species occurred at a site, then we took the cumulative abundance of those species. We then considered whether this relationship was best described a linear least squares regression or a polynomial regression by comparing the adjusted r^2 values for each fit.

Results

Are abundance and species density correlated with environmental temperature?

Species density (the number of species per site) ranged from 1-22 (mean = 9.44), and abundance (the total number of occurrences) per site ranged from 2-140 (mean = 49.5). Abundance (r^2 = 0.34, P < 0.001; Fig. 1a) (all tables and figures are located in the appendix) and species density (r^2 = 0.47, P < 0.0001; Fig. 1b) both declined as mean annual temperature (MAT) declined.

Does thermal tolerance predict range size and species density as would be the case if temperature were the sole driver of species occurrence?

We first asked whether species with broader thermal ranges had broader elevational ranges and higher elevational midpoints, as would be predicted if temperature were an important factor determining range sizes of species. We found a positive relationship between elevational ranges and thermal ranges (r^2 = 0.48, P = 0.001, Fig. 2a) as well as a positive relationship between elevational midpoints and thermal ranges (r^2 = 0.38, P = 0.004, Fig. 2b). Species with broader thermal ranges occurred at more elevations and tended to have higher elevational midpoints. We stress that these were lab-measured thermal tolerances and not simply the temperatures of sites at which species where collected.

To determine if physiological thermal limits of species alone could predict species density, we examined the relationship between the environmental conditions at each site and the composite thermal ranges of species found at that site. In comparing sites, the thermal limits of species within sites declined with mean annual temperature (CT_{max} = r^2 = 0.66, P < 0.0001, Fig. 3a; CT_{min} = r^2 = 0.67, P < 0.0001, Fig. 3b). So, species occurring at the warmest sites had, on average, the highest CT_{max} values (Fig. 3a) and species occurring at the coldest sites had, on average, the lowest CT_{min} values (Fig. 3b).

It is common to interpolate the sites at which a species could occur based on its upper and lower elevations. Here we do something similar; we interpolate the sites at which a species could occur based on its thermal limits. We assume a species can occur at all sites along the gradient within its physiological thermal range (where MAT of the site is higher than its CT_{min} but lower than its CT_{max}). When we performed this interpolation, we found that at lower temperatures, observed richness more closely matched expected richness based on thermal constraints alone; however, at warmer temperatures, there was more deviation in observed richness from the null expectation (Fig. 4), indicating that at low elevations, temperature is not the sole driver of species density.

Do species co-occur less among assemblages than would be expected if temperature alone limits membership?

When all 29 sites along the gradient were considered, species co-occurred much less than expected by chance (i.e., they were strongly segregated; observed C-score = 12.66; simulated C-score = 10.92; SES = 7.81; P < 0.0001). However, when we examined co-occurrence patterns at the warm, low-elevation sites (<1000m) and cold, high-elevation sites (>1000m) separately, we found that species in low-elevation sites were significantly segregated among assemblages

(SES = 2.06; P = 0.02), but species in high-elevation sites showed no significant deviation from randomness with respect to one another (SES = 0.94; P = 0.17).

Does competition by dominant species affect species density among assemblages?

The relationship between species density and relative abundance of dominant ants across all 29 sites was best described by a unimodal (quadratic) regression ($r^2_{adjusted}$ = 0.49, P < 0.0001 for quadratic fit vs. $r^2_{adjusted}$ = 0.21, P = 0.0007 for linear fit; Fig. 5) and was independent of a single data point ($r^2_{adjusted}$ = 0.49, P < 0.0001 for quadratic fit vs. $r^2_{adjusted}$ = 0.42, P = 0.0001 for linear fit).

DISCUSSION

Biotic and abiotic factors interact to shape spatial variation in the distribution, abundance, and diversity of ants along this extensive environmental gradient. Importantly, the influence of biotic interactions relative to abiotic factors shifts with elevation and environmental conditions. Such a finding supports the notion that the processes shaping community structure are context-dependent, and that both biotic and abiotic factors interact to determine the distribution and density of species among assemblages.

Temperature is correlated with total abundance and species density, and temperature (especially cold temperature) likely limits the ranges of species as well. Species with broad elevational ranges also have broad thermal ranges. But, we need to elucidate *why* temperature matters. In this case we can rule out some temperature-dependent mechanisms as an influence on patterns of diversity. One such influence includes the Metabolic Theory of Ecology, which depends on temperature-dependent activation energies (Brown *et al.*, 2004), as previous work with this system (Sundqvist *et al.*, 2013) and others (Hawkins *et al.*, 2007; McCain & Sanders, 2010) has demonstrated. Similarly, the pattern of ant

diversity here probably does not arise because of variation in *in situ* temperature-dependent speciation rates, since none of the studied species are endemic to the study region. In addition, temperature and net primary productivity (NPP) are not correlated in space in this system, and NPP is weakly and negatively, rather than positively, correlated with ant diversity (Sundqvist *et al.*, 2013). Finally, although it has been suggested that one effect of temperature on ant diversity is via the effects of temperature on ant foraging and access to resources, recent experimental manipulation in this same ant study system found that changes in temperature did not limit access to resources by ants (Lessard *et al.*, 2011).
We argue that stressfully low temperatures limit abundance, and in turn, species density at high-elevation sites. Specifically, underlying physiological constraints exert a filter on community membership by allowing only certain cold-tolerant taxa to establish and persist at high elevations, as low temperatures limit both overwintering success as well as slow the rate of brood development. Previous work in this system (Machac *et al.*, 2011) found that assemblages at high-elevation sites are characterized by the presence of fewer and clustered lineages, as might be expected if only the species of the restricted subset lineages with the ability to tolerate cold climates persist at high elevations. Our measurements of physiological tolerance lend support to the conclusions from previous community phylogenetics approaches. On average, populations at high-elevation sites tended to have lower CT_{min} temperatures than did populations at warmer low-elevation sites. In fact, thermal breadth also increased with elevation, suggesting that communities at higher elevations consist of individuals that can withstand a wider range of environmental temperatures than low-elevation species.

We found that observed species density varied more from the densities predicted by physiological-environmental matching alone at warmer, but observed densities in colder and more stressful conditions approximated expected

densities. Populations at higher elevations (and latitudes) often persist in areas that are colder than would be expected based on their CT_{min} (Sunday *et al.*, 2012) and low-elevation (and low-latitude) populations can persist in regions that are much warmer than their CT_{max} would suggest. Here, we found that populations in high elevation assemblages occur at temperatures that are colder than would be expected given their thermal tolerances; this has been referred to as "overfilling" the thermal niche space (Sunday *et al.*, 2012). In contrast, populations at low elevations do not occur in all of the places they might, based on their thermal tolerances alone, which has been dubbed "underfilling" of thermal niche space. It has been suggested that overfilling of niche space is due to winter survival mechanisms of physiological cold tolerance and behavioral avoidance strategies (e.g., diapause) (Diamond *et al.*, 2012). That seems likely in our case, though we did not specifically examine any potential overwintering mechanisms.

Underfilling of thermal niche space in warm sites might be due to interspecific interactions, as has often been suggested in the literature (Sunday *et al.*, 2012). Here, our null model approach lends support to the idea that interspecific interactions, especially in warm sites, limit community membership. The idea that interactions structure ant assemblages is not new (Andersen, 1992; Cerdá *et al.*, 1997; Cerdá & Retana, 1998; Bestelmeyer, 1997; Lessard *et al.*, 2009; Parr & Gibb, 2010). In fact, the strongest evidence for the effects of competition on ant assemblages comes from the collapse of many ant assemblages in the face of competitively dominant invasive species (Holway *et al.*, 2002), non-random patterns of co-occurrence among assemblages (Gotelli & Arnett, 2000; Sanders *et al.*, 2003), temporal, spatial, and resource partitioning within assemblages (Cros *et al.*, 1997; Albrecht & Gotelli, 2001; Sanders & Gordon, 2003) and the influence of competitively dominant native species (Parr *et al.*, 2005; Parr, 2008). Here, we focused on the co-occurrence of native species, and used observed

interactions as well as the C-score of Stone and Roberts (1990) and null model analyses to show evidence of the role of biotic interactions within communities. When we examined the warmer sites (those below <1000m elevation) and the colder sites (those above >1000m in elevation) separately, we found evidence for the signature of interspecific interaction in low-elevation sites, but not high-elevation sites. That is, species co-occurred less than expected at low-elevation sites, as would be predicted if competitive exclusion structured communities (Gotelli *et al.*, 2010) at high-elevation sites, species co-occurred randomly with respect to one another. These null models alone do not directly implicate interactions, but they are in agreement with three other independent lines of evidence. First, community phylogenetic evidence points to the role of interspecific competition in shaping low-elevation but not high-elevation sites (Machac *et al.*, 2011). Second, the relative abundance of competitively dominant species is highest in warmest, low elevation sites; not incidentally, those are the assemblages that dominant species influence most. Lastly, there is more deviation from the null expectation in low-elevation assemblages based on physiology-environment associations alone.

CONCLUSIONS

One of the fundamental tenets of biogeography is that abiotic and biotic factors interact to shape the distributions of species and the organization of communities, with interactions being more important in benign environments, and environmental filtering more important in physiologically stressful environments (MacArthur & Levins, 1967; Weiher & Keddy, 1995). Null models and community phylogenetic studies at large spatial scales, and manipulative experiments at small spatial scales, have hinted at such a scenario (Graham *et al.*, 2009). Taken together, our results, using a combination of observational data, null models, and physiological measurements, provide a strong test that interspecific interactions drive the distributions and density of species in warm climates, but that

physiologically driven environmental filtering predominates at high-elevation sites.

Our results also have implications for predicting the responses of biodiversity to ongoing climate change. Recent forecasts of biodiversity change in response to climate change rely on matching the thermal tolerances of species to thermal environments in the future, and most show that species in the warmest places are the most susceptible to ongoing warming because species are operating close to their thermal maxima, so any increase in temperature essentially pushes these species over the thermal edge (Deutsch *et al.*, 2008). While some studies have pointed out that organisms in the warmest places can modulate their behavior to escape stressfully high temperatures, they have generally overlooked the fact that these warm places are also where organisms are likely to face the most negative consequences of interspecific interactions. For instance, our thermal constraints models showed that diversity varied most from our expectation in the warmest places, because that is also where biotic interactions among species are the most important in limiting community membership. So, while positive interactions among species might buffer species in the face of climate change, negative interactions such as competition, might exacerbate the effects of climate change on biodiversity in warm environments. Models that focus on the future of biodiversity in warm environments, where most of biodiversity is, should also examine the combined and relative effects of biotic interactions and abiotic constraints and how these processes scale up to influence patterns of diversity.

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APPENDIX I

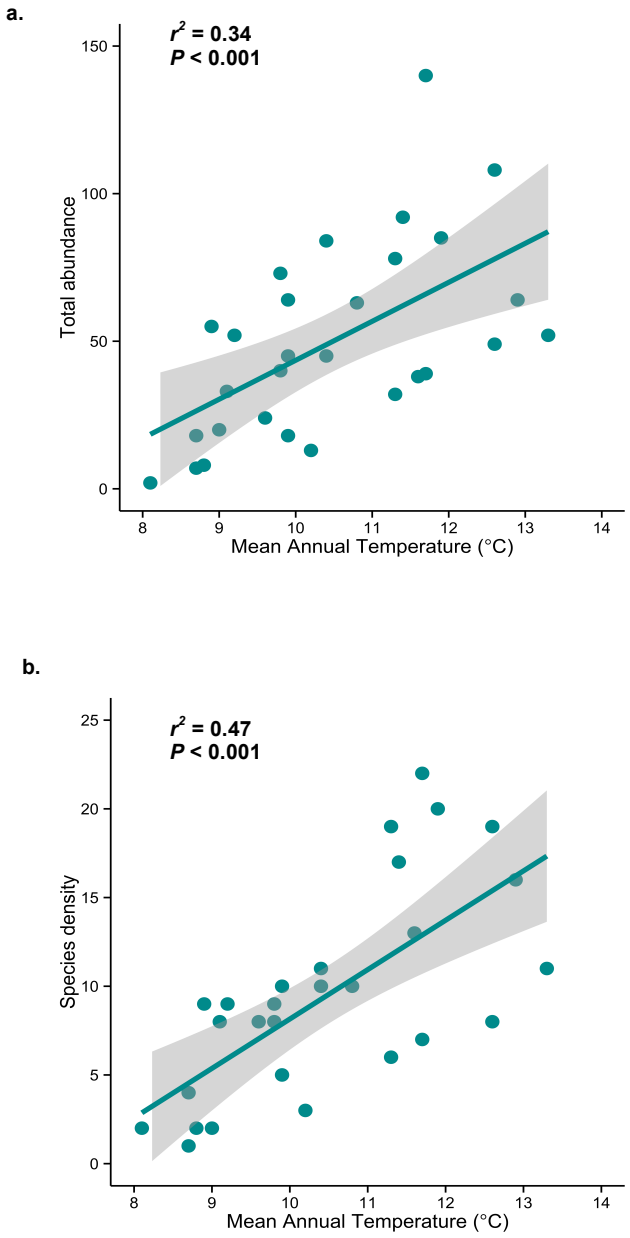

Figure 1. The relationship between (a) total abundance and temperature and (b) species density and temperature shows increasing abundance and density with increasing mean annual temperatures. Temperatures are current (~1950-2000) mean temperatures for each site extrapolated from the WorldClim database (Hijmans *et al.*, 2005) at a resolution of 30 arc-seconds. The line in each figure is the best-fit linear regression and the shaded area is the 95% Confidence Intervals.

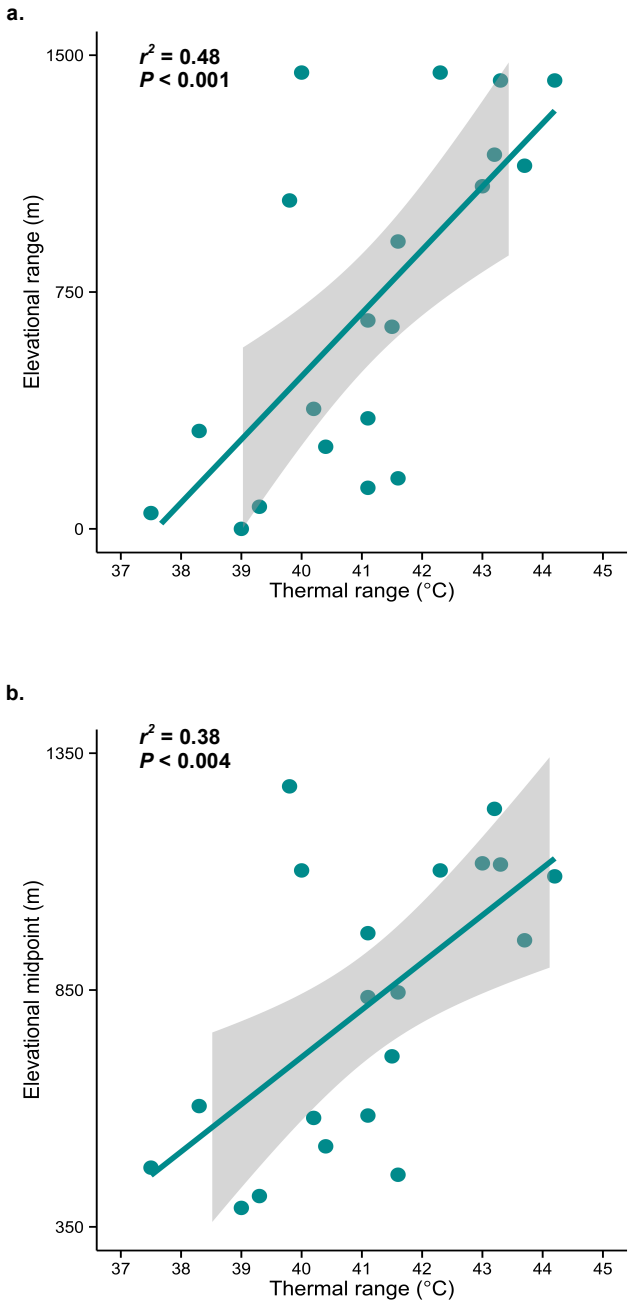

Figure 2. Thermal ranges (CT_{max} – CT_{min}) with 95% Confidence Intervals show a positive relationship with (a) elevational ranges (r^2 = 0.48, *P* = 0.001) and (b) elevational midpoints (r^2 = 0.38, *P* = 0.004) of 20 species for which we obtained both physiological and distributional data. Elevational ranges were calculated as the highest elevation at which a species was recorded minus the lowest elevation at which a species was recorded.

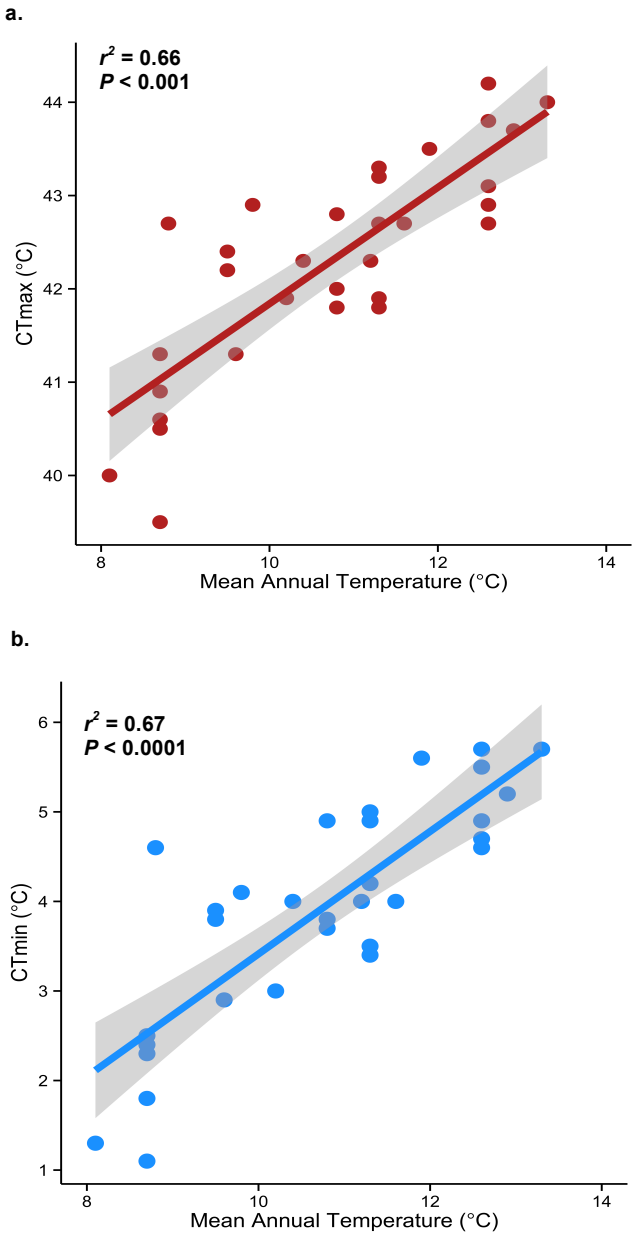

Figure 3. Species that occur at the warmest sites have, on average, (a) the highest critical thermal maxima (CT_{max}) values and (b) those that occur at the coldest sites have, on average, the lowest critical thermal minima (CT_{min}) values. Each point is the mean of the thermal limits for all species averaged for each site. The line and shaded area in each figure is the best-fit linear regression and 95% Confidence Intervals, respectively. Temperatures were extrapolated from WorldClim (Hijmans *et al.*, 2005) at a resolution of 30 arc-seconds and represent mean temperatures from ~1950-2000.

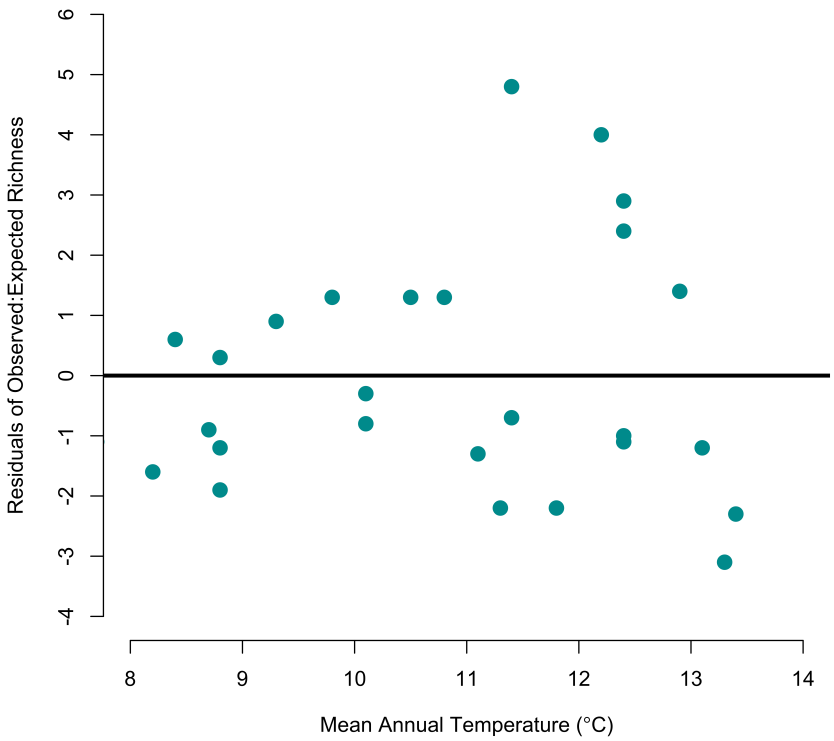

Figure 4. Residuals of the observed and expected species density based on thermal overlap in physiological limits and environmental temperatures. At lower temperatures, observed richness more closely matches expected richness; however, at warmer temperatures, there is more deviation in observed richness from the null expectation.

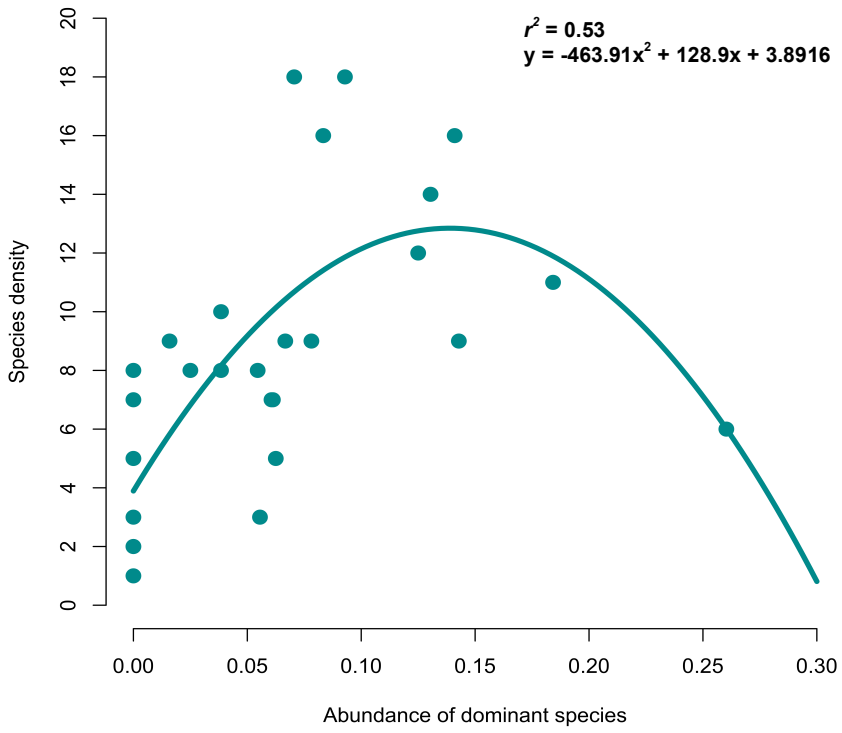

Figure 5. The relationship between the number of species in an assemblage (not including the dominant species, if present) and the abundance of one of the competitively dominant species. The abundance of both dominant and subordinate species increases until the dominant species begin to limit the abundance of subordinate species.

CHAPTER II
ECOLOGY AND EVOLUTION SHAPE THE THERMAL NICHE

Coauthored by Andrew D. Nguyen, Clint Penick, Lauren Nichols, John Stanton-Geddes, Sarah E. Diamond Robert R. Dunn, Aaron M. Ellison, Nicholas J. Gotelli, Sarah Helms Cahan, and Nathan J. Sanders

ABSTRACT

Climate change is altering species distributions through rapid increases in both the mean temperature and increased variability in temperatures. Species respond in diverse ways to warming, but we know little about variation among populations of the same species, or intraspecific variation, in response, or the responses of sister species. How species respond to these changes depends in large part on their physiology, which also determines their current distributions. Species with wide-ranging distributions tend to be able to tolerate broad temperature ranges, which may make them less susceptible to the effects of warming than small-ranged species, which typically cannot tolerate broad temperature ranges. Here, we focus on the physiological tolerances of sister species along a geographical cline in the eastern U.S. and conduct a common garden experiment to determine acclamatory and adaptive responses to warming. We found that, regardless of acclimated rearing temperatures, ants maintained a geographic cline in thermal limits and sister species differed in their responses to temperature. Taken together, these patterns suggest that thermal limits may be constrained and that local adaptation may limit the responses of ants to climatic warming.

INTRODUCTION

Climate change is altering and will alter species distributions through rapid increases in both the mean temperature and increased variability in temperatures. How species respond to these changes depends in large part on their physiology, which also determines their current distributions. Species with wide-ranging distributions tend to be able to tolerate broad temperature ranges, which may make them less susceptible to the effects of warming than small-ranged species, which typically cannot tolerate broad temperature ranges (Deutsch, et al. 2008).

Range size relates to the degree of environmental temperature variation that species experience and should match a species' physiological traits. For example, greater environmental temperature variation selects for species that have larger thermal breadths, (i.e. a greater range between their upper (CT_{max}) and lower (CT_{min}) thermal limits) (van Berkum, 1988). However, species can deviate from this prediction if selection does not operate on these traits equally, or at all (Huey and Kingsolver, 1993). Physiological traits are not static, but rather can change within a generation (acclimation) and across generations (adaptation), which together may temper the effects of warming. In order to improve predictions about how biodiversity will respond to ongoing warming, it is important to understand whether the responses of particular populations arise from acclimation or selection.

Most of the work to date that links physiology, range dynamics, and climate change has focused on the differences among species. Species respond in diverse ways to warming, but we know little about variation among populations of the same species, or intraspecific variation, in response, or the responses of sister species. Recent innovations in sequencing allow for population level

analyses of thermal traits. Variation in the responses of populations to warming is required if there is to be an evolutionary response, yet organisms living close to their thermal limits (specifically, CT_{max}) will have limited acclimation ability in relation to climate change (Stillman, 2003; Deutsch *et al.*, 2008) and previous studies have shown that extending upper thermal limits has low adaptive potential (Kellermann *et al*., 2012). In fact, CT_{min} has more additive genetic variation than CT_{max}. Additionally, behavioral thermoregulation may play a large role in determining acclamatory or evolutionary effects. Organisms that are effective thermoregulators can ameliorate detrimental effects of climate warming and may, over time, shift their thermal performance (Angilletta, 2009). However, patterns of thermoregulatory behavior and thermal acclimation are variable and can lead to lasting changes in thermal sensitivity (Bayne *et al.,* 1977; Niehaus *et al.,* 2012), as well as induce energetic tradeoffs (Angilletta, 2009). To estimate evolutionary and acclamatory responses to warming, a biogeographical approach is needed that examines not only the variation among species, but also accounts for the variation within species.

This study combines field-collected and common garden assessments of physiological limits to examine the geographic variation in response to warming in ants. First, we determined the degree to which local environmental conditions matched physiological limits (CT_{max} or CT_{min}) by assessing intra-specific (e.g., population-level) variation in thermal tolerance on field-collected samples across a latitudinal gradient. Second, we tested the effect of acclimation on CT_{max} and CT_{min} by rearing whole colonies collected from the same latitudinal gradient under two temperature regimes in a common garden experiment. Finally, we used recent innovations in sequencing techniques to examine population-level variation in thermal traits. Ants are a good model taxon to examine local adaptation and geographic clines in response to warming, as they are a ubiquitous and ecologically important taxon. Specifically, ants within the genus

Aphaenogaster are an ideal study system for measuring local adaptation, as they have a large geographic distribution and has therefore experienced historical climatic shifts. Additionally, *Aphaenogaster* ants have been shown to exhibit thermal sensitivity (Diamond *et al.,* 2012a), as well as temperature-dependent species differences in phenology (Warren *et al.*, 2011), activity (Stuble *et al.,* 2013), foraging and seed dispersal (Pelini *et al.*, 2011a; Warren *et al.*, 2011; Warren & Bradford, 2013; Stuble *et al.*, 2014), and physiology (Warren & Chick, 2013).

METHODS

Field collections

Between May and July 2012, we collected individual workers of the widespread genus *Aphaenogaster* along a latitudinal gradient in the eastern United States extending from Florida (29.6557 N, -82.2765 W) to Maine (45.8935 N, -69.0491 W) (Fig. 6) (all tables and figures are located in the appendix). Collection was conducted across an extensive thermal gradient to encompass a considerable amount of the latitudinal range of the genus. We excluded sites outside the range of those collected the following year to maintain a similar sampling area as the common garden. Workers collected in 2012 were not included in the common garden experiment, but rather were subjected to physiological tests (described below) within 6 hours of field collection to minimize any potential acclamatory response.

Common garden

The following year (April through July 2013), we collected ant colonies (including workers and a queen, henceforth, queenright) along a similar geographic gradient from Georgia (32.8807, -81.9572) to Maine (44.9818, -68.5174) (Fig. 6) by sampling deciduous and mixed-hardwood forests. We standardized colony size to 1 queen and 100 workers and acclimated colonies to laboratory

conditions (~25°C and standard long-day photoperiod) for two weeks prior to beginning two different temperature treatments at North Carolina State University. We haphazardly selected colonies from different sites and assigned them to a temperature treatment of either 20 ± 2°C (24 colonies) or 26 ± 2°C (19 colonies). We maintained colonies on a 14h:10h light-dark cycle and fed an ant specific artificial diet (Bhatkar and Whitcomb, 1970) supplemented with beetle larvae for 6-8 weeks. Colonies were also provided 20% sucrose solution in vials plugged with cotton. We housed colonies in artificial nest boxes with a plaster floor that was moistened each day to maintain adequate humidity. Nest chambers were covered with a Plexiglas square. For full common garden experimental design, see Penick *et al*. (*in review*).

Thermal tolerance

In both field-collected and lab-reared ants, we used the loss of righting response as the measure of thermal tolerance and conducted physiological testing using glass test tubes housing individual ants in a refrigerated water bath (Ac-150-A40, NesLab, ThermoScientific). We increased (or decreased for CT_{min}) temperatures by $1°min^{-1}$ until thermal limits were reached as described in Warren and Chick (2013). We tested 10 individuals from each colony from each site (5 workers for CT_{max} and 5 workers for CT_{min}) and report the thermal limits as the means of the 5 individuals tested, respectively. In total, we tested 1040 *Aphaenogaster* ants combined from 49 sites (field-collected ants) and 43 colonies (common garden ants). Voucher specimens from each colony are deposited at North Carolina State University.

To determine whether local environmental conditions shape thermal traits, we extracted mean annual temperature (MAT) and seasonality from the publically available Worldclim (Hijmans *et al.* 2005) at a resolution of 30 arc-seconds.

Library preparation

We preserved whole ants at either -20°C or in 95% ethanol at room temperature. We then extracted genomic DNA from tissue from a single worker from each colony (100 individuals total) with the Qiagen DNAeasy kit according to the manufacturer's instructions. We homogenized ant tissue prior to extraction with ~20 1.4mm zirconium silicate beads in 200ul chilled ATL buffer for three minutes in a Next Advance Bullet blender at maximum speed. The 39 experimental colonies were genotyped as part of a set of 48 individually-barcoded ddRADseq libraries constructed from 100-200ng of genomic DNA per individual. Briefly, samples were double-digested with the restriction enzymes NlaIII and MluCl at 37°C for three hours, purified using a 1.5X concentration of AMPure purification beads, and quantified with a Qubit analyzer. We ligated the purified samples to barcoded P1 and universal P2 adaptors, and normalized sample concentrations by pooling 40ng of each sample. We purified three hundred microliters of the pooled library with a 1X AMPure bead purification eluted into 30ul of Qiagen AE buffer. The ligated fragments were amplified in seven 20ul PCR reactions containing approximately 20ng of DNA with the Phusion Taq PCR kit. We empirically determined the appropriate number of PCR cycles by comparing amplification intensities of 11, 13, and 15 cycles; the final library was constructed using 13 cycles. The combined PCR reactions were pooled and purified with 1.5X AMPure beads into a final volume of 30ul. We size-selected fragments 300-400bp in total length from a 1.5% agarose gel and extracted with the QIEX II gel extraction kit. We verified library size range and quality on a Bioanalyzer and with kapa qPCR. The library was single-end sequenced in a single HiSeq 2000 rapid-run lane at the University of Vermont Advanced Genome Technologies Core facility, yielding approximately 2.5 million reads per sample.

Bioinformatics

Sequences were demultiplexed using the program sabre (https://github.com/najoshi/sabre), allowing for up to a single base pair mismatch,

and the restriction site sequence was trimmed. We trimmed the total length of all sequences to 90bp and completely excluded low-quality reads, defined as those whose quality score dropped below 10 at any point along the sequence, from downstream analysis.

Because there is no sequenced genome available for the genus *Aphaenogaster* or closely related ant genera, we used a subset of five samples from across the geographic extent of the transect (Table 1) to identify a repeatable subset of loci showing Mendelian inheritance patterns, which was then used as a reference against which the complete sample set was mapped and genotyped. The reference sample sequences were assembled into homologous tags using the denovo.pl pipeline in STACKS, and those tags for which a) there were from zero to three SNPs present across the five samples, b) all five samples contained one or more reads at the tag, c) all SNPs were biallelic, and d) all samples contained no more than two alternate haplotypes, were retained. We assembled the consensus sequences of the 61,518 retained tags into a fasta reference file and the filtered sequence reads of all samples, including the reference samples, were mapped against the reference with Bowtie. We identified SNP genotypes by assembling the mapped reads into stacks using the ref_map.pl pipeline in STACKS. For each sample, we concatenated the SNP genotypes across tags into a single pseudo-sequence that was used for all downstream biogeographic analyses.

RESULTS

Along the latitudinal cline, sister species of *Aphaenogaster* responded differently to temperature. CT_{max} of *A. picea* did not vary with MAT ($r^2 = 0.05$, $P = 0.13$; Fig. 7), yet CT_{min} declined more rapidly with decreasing MAT ($r^2 = 0.89$, $P < 0.001$; Fig. 7). This results in *A. picea* having a broader thermal breadth at higher

latitudes and elevations (i.e. lower MAT). Interestingly, this pattern is reversed for *A. rudis* (CT_{max}: $r^2 = 0.84$, $P < 0.001$, CT_{min}: $r^2 = 0.74$, $P = 0.003$; Fig. 7).

Both PGLS and Eigen function analyses found significant effects of phylogeny and ecology for CT_{max}, but not for CT_{min}. In the PGLS analysis for CT_{max}, MAT was a significant predictor, even in the presence of a large phylogenetic signal (lambda= 0.83). For CT_{max}, there was also a significant effect of MAT and no evidence of phylogenetic signal (lambda=0). Eigen function analysis produced four principle components, which capture 77.7% of the variation within the phylogeny, and identifies different nodes of the phylogeny. PC1 explains 56.28% of the variation and represents the split between *A. picea* and *A. rudis*. PC2 represents the split between two clades within *A. rudis* and explains 13.37% of the variation in the phylogeny.

In the full model for CT_{max} responses, there were no significant main effects, but there was an interaction between PC1 (representing *A. rudis*/*A. picea* split) and MAT (B=1.535, p<0.01). In the full model for CT_{min} as the response, there was a significant main effect of MAT (p<0.001) and PC1 (p<0.001), and a significant interaction between them (B=-1.78, z value = 3.96, p<0.0001). Due to the interaction between MAT and PC1 (phylogeny), we performed additional regressions to determine the relationship between thermal traits (CT_{max} and CT_{min}) and MAT for each species. For *A. picea*, there was no significant effect of MAT, rearing temperature, or phylogeny on CT_{max}, but there was a significant positive effect of MAT on CT_{min} (B = 0.885, z value= 14.542, p<0.0001). For *A. rudis*, there was a positive effect of MAT on both CT_{max} (0.410, z value = 4.71, p<0.0001) and CT_{min} (0.280, z value = 3.58, p<0.001).

There was no effect of lab acclimation on thermal limits (Fig. 8). *Aphaenogaster* ants in the common garden exhibited similar intra-specific variation in thermal

limits as documented in the field, regardless of lab rearing temperature. Ants collected and tested directly from the field exhibited more variability in CT_{max} than those reared in the common garden.

We recovered highly supported relationships among colonies that follow a southern to northern split in their geographic range. *A. rudis* is secluded to the southern end of the U.S., while *A. picea* occupies the northern end and both represent monophyletic clades and *A*. *fulva* is sister to *A. picea* and *A. rudis* (Fig. 9).

DISCUSSION

We reconstructed phylogenetic relationships reflecting the geographic distributions of a southern (*A*. *rudis*) and northern (*A. picea*) clade of ants with some range overlap. When accounting for these relationships in our phylogenetic analyses, we found patterns in thermal limits that suggest local adaptation. The northern clade, *A. picea,* exhibited more variation in CT_{min} than the southern clade of *A. rudis*, but the *A. rudis* clade exhibited more variation in CT_{max}. In fact, there was no clinal variation in CT_{max} for *A. picea*, suggesting little or no selection pressure on this physiological trait. This response could be due to differing selective pressures facing these two sister species. *Aphaenogaster picea* is considered to be a cold-adapted species, as it has earlier spring emergence and a lower CT_{max} and CT_{min} as compared to the *A. rudis* clade (Warren *et al.,* 2011; Warren & Chick 2013). Additionally, *A. picea* occurs in cooler habitats than the *A. rudis* clade and may rarely (or never) experience temperatures close to its CT_{max}, whereas species within the *A. rudis* clade occur and forage at temperatures closer to its CT_{max} and would therefore have a more narrow estimate of warming tolerance (the difference between CT_{max} and mean environmental temperature) (Deutsch *et al.*, 2008). The differences in how selection shaped these thermal limits is somewhat consistent with the concept known as Rapoport's Rule.

Rapoport's Rule posits that the ranges of species are larger at higher latitudes than at lower latitudes due to more variation in the thermal environment. *Aphaenogaster picea* follows the rule because the thermal niche breadth increased with latitude; however, *A. rudis* had a larger thermal breadth at lower latitudes, reversing the rule.

There was no effect of rearing temperature on the thermal tolerance of *Aphaenogaster* ants; however, ants collected and tested directly from the field have more variability in thermal limits than those reared in the common garden. This could be due to the physical condition of the ants. Ants collected from the field were workers that were actively foraging at the time of collection, while those tested from the common garden were provided with a constant food source and did not need to forage great distances or for long periods of time. Common garden ants therefore could have been in better physical condition than some of those collected from the field. Regardless of this variation, there was no effect of acclimation on *Aphaenogaster* ants. This lack of evidence for lab acclimation may indicate that thermal limits are more genetically constrained than previously thought and that *Aphaenogaster* may have limited acclimation ability in relation to climatic warming. Additionally, cold tolerance seemed to be less constrained than warm tolerance. The slope for CT_{min} for *A. picea* is greater than the slope in CT_{max} for *A. rudis*, but we cannot rule out greater selection pressure for CT_{min}.

Through our amalgam of techniques and analyses using field lab experiments as well as phylogenetic components and genetic sequencing, we can propose that both ecology and evolution shape thermal traits in this widespread genus of ant. Species within the genus *Aphaenogaster* are susceptible to warming, but for different reasons. *Aphaenogaster picea* may be susceptible to rapid warming because there is little variation in CT_{max}, whereas ants in the *A. rudis* clade may be more susceptible because they operate closer to their thermal limits (Deutsch

et al., 2008; Diamond *et al.*, 2012b). Both species lack acclimation ability which may also result in greater climate warming susceptibility overall. *Aphaenogaster* ants are keystone mutualists that disperse approximately 90% of understory plants, many of which are eliaosome-containing seeds (Zelikova *et al.,* 2008; Ness *et al.*, 2009). Climatic warming will not be uniform across the landscape; therefore populations will experience varying degrees of warming. Populations that are unable to acclimate or adapt to a rapidly changing thermal environment may face decreases in colony sizes and overall local abundance. Thus, local extirpations or range shifts of a key seed disperser will likely cause mismatches in ant-plant mutualisms and have negative cascading effects on deciduous and mix-hardwood ecosystems.

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APPENDIX II

Table 1. Samples collected in the summer of 2013 in a latitudinal transect from Georgia to Maine for a common garden experiment.

Sample	Species	Site	Latitude °N	Longitude °W	Rearing temperature (°C)
01B	Aphaenogaster rudis	MagSpr4	32.8795	81.9571	20
02B	Aphaenogaster rudis	HW5	33.5556	81.7338	26
04C	Aphaenogaster rudis	UNF1	35.3693	79.9745	26
05B	Aphaenogaster rudis	GSMNP 4	35.6363	83.4938	20
05D	Aphaenogaster picea	GSMNP 5	35.6367	83.493	20
07A	Aphaenogaster rudis	BRP2	35.9264	81.9538	26
07B	Aphaenogaster rudis	BRP9	35.9264	81.9538	20
08A	Aphaenogaster rudis	Ijams6	35.9557	83.864	20
08D	Aphaenogaster rudis	IJams1	35.9568	83.8668	26
09A	Aphaenogaster rudis	RC12	36.0364	79.0772	20
10A	Aphaenogaster rudis	LVA9	37.4211	79.181	20
10B	Aphaenogaster rudis	LVA12	37.4211	79.181	20
11A	Aphaenogaster rudis	WP9	39.7255	76.079	26
13A	Aphaenogaster picea	HSP6	41.0226	75.71777	20
13B	Aphaenogaster picea	HSP7	41.0226	75.71777	20
13C	Aphaenogaster picea	HSP9	41.0213	75.7173	26
13D	Aphaenogaster picea	HSP12	41.0219	75.7172	26
15A	Aphaenogaster picea	DSF4	41.298	75.0112	20
15D	Aphaenogaster picea	DSF12	41.3044	75.0093	26
16A	Aphaenogaster picea	BRM4	41.4041	74.0209	20
16B	Aphaenogaster picea	BRM8	41.404	74.0219	20
17A	Aphaenogaster picea	Bard10	42.0174	73.9163	20
17B	Aphaenogaster picea	Bard9	42.0177	73.9159	20
19A	Aphaenogaster picea	HF001	42.5628	72.2319	20
20A	Aphaenogaster picea	APB10	42.7184	73.8561	20
20C	Aphaenogaster picea	APB3b	42.7197	73.8566	26
20D	Aphaenogaster picea	APB8	42.7185	73.8561	26
21A	Aphaenogaster picea	Bear6	43.0993	71.3481	20
21B	Aphaenogaster picea	Bear5	43.0993	71.3481	20
21C	Aphaenogaster picea	Bear3	43.0993	71.3481	26
22B	Aphaenogaster picea	SEB8	43.9237	70.5828	20
22C	Aphaenogaster picea	SEB9	43.9239	70.5837	20
23A	Aphaenogaster picea	MM1	44.1111	71.1403	26

Table 1. Continued

Sample	Species	Site	Latitude °N	Longitude °W	Rearing temperature (°C)
26A	Aphaenogaster picea	MB1	44.5	72.64	20
26D	Aphaenogaster picea	MB2	44.5	72.64	26
26E	Aphaenogaster picea	MB6	44.5	72.64	26
27A	Aphaenogaster picea	KBH4b	44.5676	69.9214	26
28B	Aphaenogaster picea	Brad6	44.9818	68.5174	26

* Denotes samples used as a reference for calling SNPs

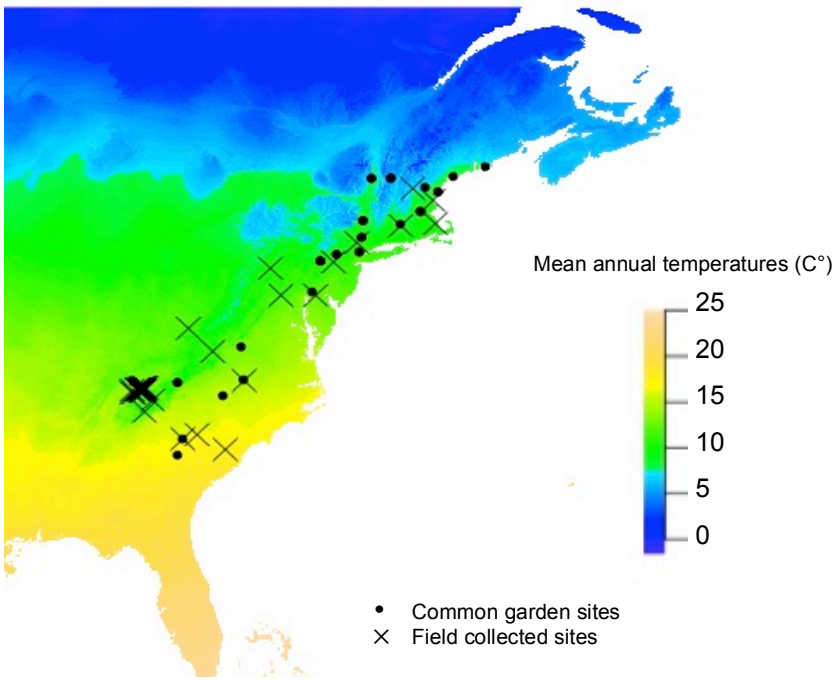

Figure 6. Site collections of *Aphaenogaster* ants. Each point represents a sampling site for the common garden experiment (circles) and field-collected thermal limits (×). Sites ranged from approximately 32.88°N to 44.98°N. Colors represent mean annual temperatures extrapolated from WorldClim (Hijmans *et al.*, 2005) at a resolution of 30 arc-seconds ranging from ~1950-2000.

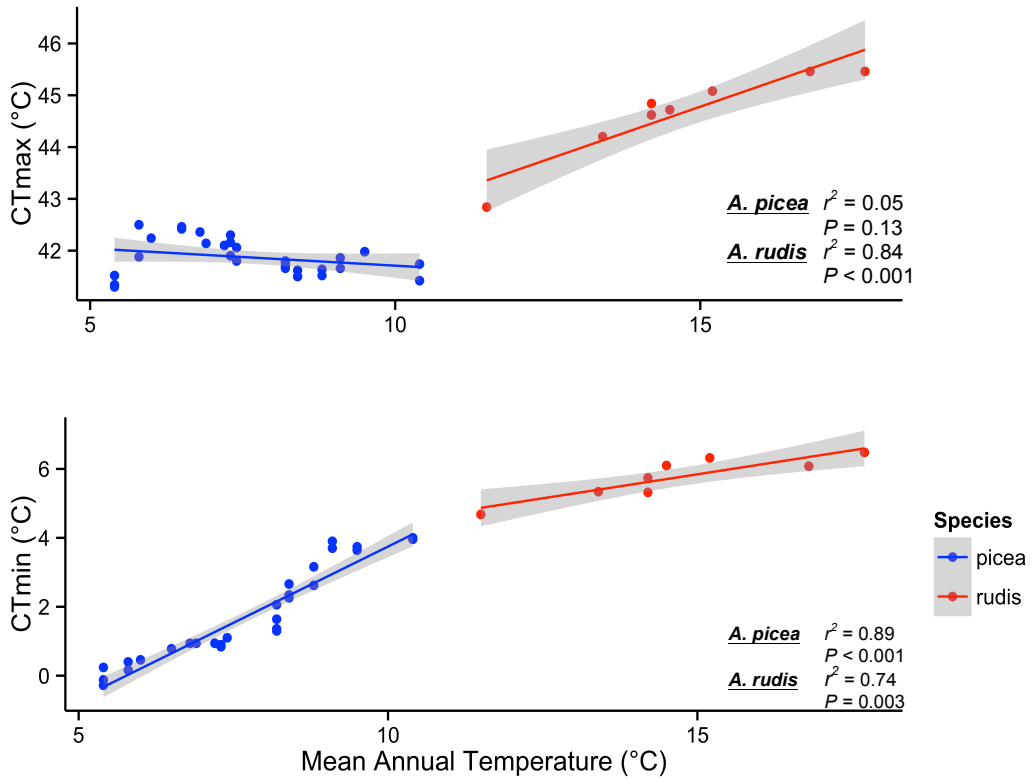

Figure 7. Thermal limits plotted against mean annual temperature (MAT) indicate differing responses to temperature among sister species of *Aphaenogaster*. There is little variation in CT_{max} with MAT for *A. picea* ($r^2 = 0.05$, $P = 0.13$), yet CT_{min} declines more rapidly with decreasing MAT ($r^2 = 0.89$, $P < 0.001$). This pattern is reversed for *A. rudis* (CT_{max}: $r^2 = 0.84$, $P < 0.001$, CT_{min}: $r^2 = 0.74$, $P = 0.003$).

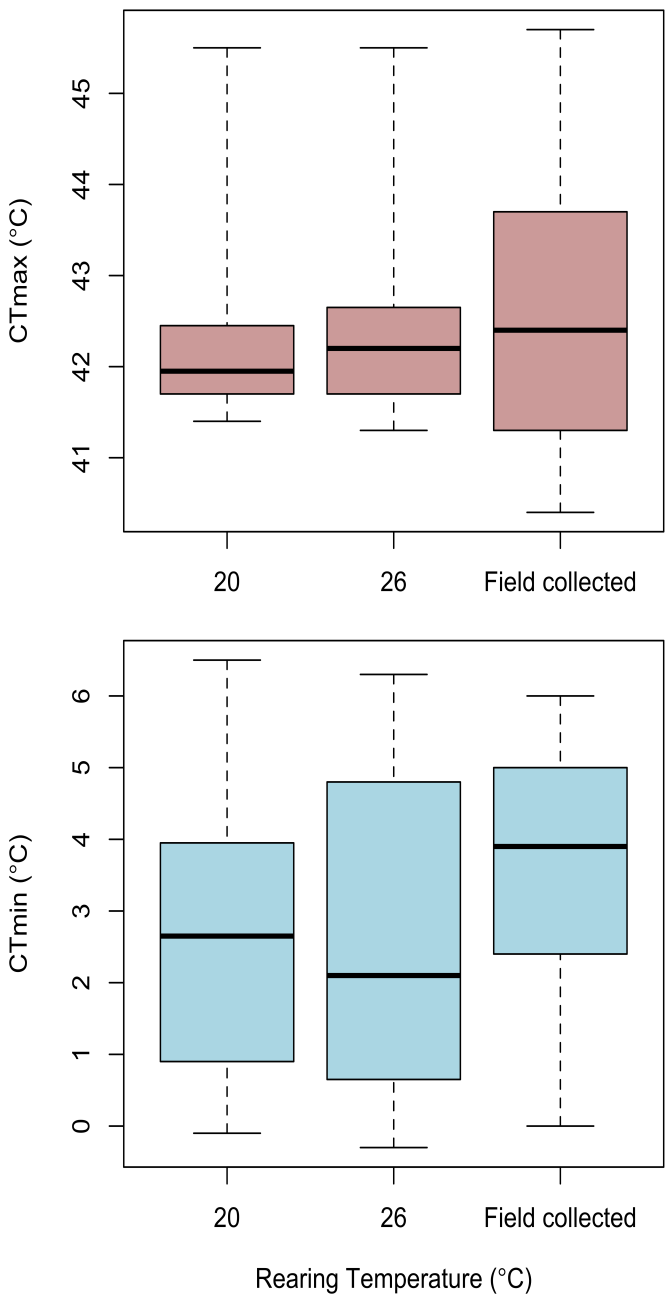

Figure 8. There is no effect of rearing temperature on the thermal tolerance of *Aphaenogaster* ants. Ants collected and tested directly from the field have more variability in thermal limits than those reared in the common garden.

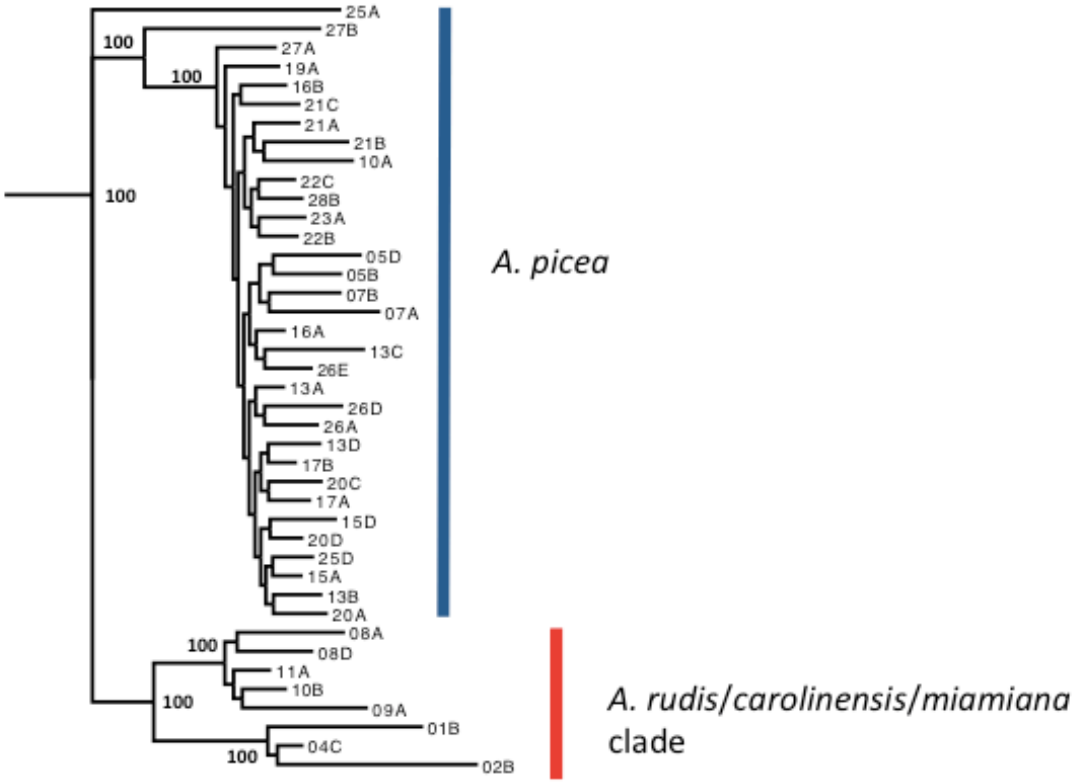

Figure 9. Relationships of populations of two different species (*A. picea, A. rudis* clade). To reconstruct phylogenetic relationships, we first assembled a 78,079 SNP matrix generated from double restriction enzyme assisted digestion-sequencing (ddRAD-seq; Peterson *et al*., 2012). This matrix was analyzed in a maximum likelihood framework in RAxML 8 (Stamatakis 2014) and group support was evaluated with 100 fast bootstrap replicates.

CHAPTER III
TIMING MATTERS: HOW RAMPING SPEED AFFECTS HEAT STRESS RESPONSES

Co-authored by Clint A. Penick, Andrew D. Nguyen, Katharine L. Stuble, and Sarah E. Diamond

ABSTRACT

Critical thermal limits (CT_{max} and CT_{min}) define the maximum and minimum temperatures an organism can withstand. Using simple techniques, thermal limits have been measured for many taxa and are increasingly used to predict the responses of species to climate change. Yet there are multiple techniques used to measure thermal limits, and each potentially yielding different results. Thus, there is debate about which techniques reveal an organism's "true" thermal limit, and which methods are the most ecologically relevant. An alternative explanation is that different methods for measuring thermal limits provide insight into separate aspects of an organism's life-history. In such a scenario, different measurements of thermal limits could identify unique thermal challenges for species that inhabit complex thermal environments. We used ants, a widespread and ecologically important taxon, to test how two measurements of CT_{max} (acclimated response and fast response) correlate with distinct aspects of the life histories of suites of species. The acclimated response is a slow ramping speed of 1°C every 5 minutes and indicates the ability of an organism to gradually adjust to warming. The fast response is a ramping rate of 1°C every one minute and indicates how thermal accumulation may limit CT_{max}. We found that the acclimated CT_{max} correlated with traits associated with the nest environment of species and varied little among populations within a species. In contrast, the fast response did not correlate with nest-based traits, but showed strong signatures of selection along a climate gradient. These results suggest something about how we quantify thermal maxima and what it means for dealing with climate change.

INTRODUCTION

Determining the impacts of climatic warming on individual taxa or collections of species has mainly focused on quantifying the ability of individuals to withstand a uniform change in the thermal environment. Despite the obvious fact that temperature is one of the primary factors impacting the performance and fitness of ectotherms (Andrewartha and Birch, 1954; Hawkins et al., 2003; Currie et al, 2004), and that temperature change rarely occurs at the same rate, we know little about how different rates of temperature increase vary among species or ontogeny. Because temperature changes as a result of ongoing climate change, it is increasingly important to understand these relationships, yet few studies have.

Critical thermal limits (CT_{max} and CT_{min}) are ecologically-relevant measures of the ability of an organism to endure exposure to high and low temperatures, respectively. These measures (specifically CT_{max}) have been used to predict and extrapolate the diversity and distributions of species in light of climate change (Kearney *et al.*, 2009; Deutsch *et al.*, 2008; Diamond *et al.*, 2012). However, there are almost as many ways to measure CT_{max} as there are organisms on which to measure it, and these different techniques can lead to different estimates of CT_{max}, leaving us with different data in the literature and therefore, different predictions about the future (Rezende *et al*. 2011; Terblanche *et al.,* 2011).

One possible solution would be to simply settle on the best method for measuring CT_{max}. But what is the best method? And which method provides the most accurate estimate of thermal performance (Lutterschmidt & Hutchison, 1997; Terblanche *et al.,* 2011)? Different ramp styles (e.g., dynamic vs. static) and

rates (fast vs. slow) can yield different estimates of CT_{max} (Terblanche *et al.,* 2011; Castañeda *et al.*, 2012) and may indicate different coping mechanisms for different life stages. Comparing different methods for measuring CT_{max} may provide insight into distinct coping mechanisms associated with thermal performance and address evolutionary implications for differences between thermal performance metrics under various warming scenarios. Alternatively, different ramping methods may provide insight into separate aspects of an organism's life history. For instance, if an organism's CT_{max} is higher using a faster ramp rate, this suggests that accumulation of thermal damage limits an organism's CT_{max}. However, if CT_{max} is higher using a slow ramp technique, acclimation may be important for achieving higher CT_{max}. Thus, each of these approaches can provide different kinds of information about how organisms deal with their thermal environment, and how they might respond to increasing global temperatures. Moreover, it might also be the case that temperature has different effects on different life history stages of organisms, e.g., 2°C of warming affects a tadpole differently than an adult frog (Murray *et al.*, 2011; Garcia *et al.*, 2014). Life history characteristics are also likely to play a significant role in how responsive certain species are to extreme events, which are contingent on magnitude (Jentsch *et al.*, 2007) and timing (Jackson *et al.*, 2009) in relation to life-history. Thus, understanding how different kinds of ramping styles and rates of ramping are used to estimate CT_{max}, and how those different kinds of ramping interact with life history, is critical to increasing our mechanistic understanding of how organisms deal with temperature and changing environments.

In social insects, environmental conditions vary throughout ontogeny. For example, in most ant species, brood stay in the nest throughout their development, and some workers spend substantial portions of their lives inside the nest (Anderson & Munger, 2003; Penick & Tschinkel, 2008). Other workers experience two distinct environments: inside the nest, and outside the nest when

foraging or performing other tasks. Ground nesting is the ancestral and most common form of habitat (Lucky *et al*., 2013), but whether above or below ground, temperatures inside the nest are buffered by nest properties and social thermoregulation, whereas foragers are exposed to greater thermal extremes that vary with latitude (Dunn *et al*., 2010), elevation (Sanders *et al*., 2007), or microhabitat (Diamond *et al*., 2013; Kaspari *et al.*, 2014). Thus, understanding how ants deal with these rising temperatures across their life history stages can enable more accurate predictions about the consequences of warming on ants and the functions and services they provide. Here, we explore the relationship between fast and slow ramp speeds as a way to assess CT_{max} among 14 ant species and consider links between ramping rate and developmental traits. Furthermore, we examine intra-specific variation in ramping rates of CT_{max} for populations of the widespread genus *Aphaenogaster* to determine if selective forces are acting on physiology along a climatic gradient.

METHODS

Sampling

We collected full colonies (workers and queens, henceforth, queenright colonies) of 14 ant species from deciduous forests around Durham, North Carolina, USA (36.03° N, -78.87° W) and the Blue Ridge Parkway (35.926° N, -81.953° W). In addition, we collected colonies belonging to the widespread *Aphaenogaster rudis* complex along a latitudinal gradient in the eastern United States extending from Georgia (32.88° N, -81.95° W) to Maine (44.98° N, -68.51° W). Species in the *A. rudis* complex are abundant in deciduous forests (King et al., 2013) and nest in a variety of habitats across extensive thermal gradients, making it a good focal group for clinal studies. We chose sites that cover a considerable amount of the geographic range of *A. rudis*, which *therefore* encompasses a large climatic range. Colonies of all species were acclimated to laboratory conditions for two

weeks prior to beginning a common garden treatment at North Carolina State University. We then reared colonies at a temperature of 23 ± 2°C for 4 weeks before beginning physiological testing. Voucher specimens from each colony are deposited at North Carolina State University.

Determining thermal limits

We measured the critical thermal maximum (CT_{max}) on 145 worker ants using two different ramping speeds to capture their acclimated response (slow ramp) and fast response (fast ramp) to increasing temperatures. The slow ramp increased at 1ºC every five minutes, while the fast ramp began at 30ºC and increased 1ºC per minute. CT_{max} was defined as the temperature at which a worker lost muscle coordination (i.e., inability to right itself after being flipped on its dorsum). The time required to reach CT_{max} using the fast ramp was less than 10 minutes in most cases, while CT_{max} was reached after 10-45 minutes using the slow ramp method. Comparisons between thermal limits were analyzed with linear regression models using mean thermal limits from 5 workers from each colony, of each species.

Finally, we also compared the relationship between slow-ramp and fast-ramp CT_{max} with species-specific differences in pupal development time at 20°C. Pupal development time was calculated for each species by dividing larvae among colonies. Larvae were held at one of four temperatures (20°, 23°, 26°, 29°C). Pupal development time was quantified as the number of days between the first appearance of pupae and the date when new workers eclosed (full methods described in Penick et al. *in review*). We tested whether there was a significant relationship between acclimated CT_{max} and fast CT_{max} with pupal development time (at 20°C) using linear regression. We used a one-tailed test due to *a priori* expectations that species with a low CT_{max} have faster development at cool temperatures than species with a high CT_{max} (Penick et al. *in review*).

RESULTS AND DISCUSSION

Species vary in their ability to withstand extreme temperatures (Diamond *et al.*, 2012b) and this variation depends on geography (Chick *et al.*, *in review*). Thermal limits may depend on different physiological mechanisms, which could differ by species, and vary between ramping speeds. While CT_{max} is among the most common metrics used to characterize differences in how species respond to temperature, we found little to no correlation between a species' CT_{max} calculated using two distinct methods (Fig. 10) (all tables and figures are located in the appendix). In some cases, species exhibited a higher CT_{max} when given time to acclimate (slow-ramp), while in other cases species exhibited a higher CT_{max} when increases in temperature were more abrupt (fast-ramp; Fig. 11). In the past, discrepancies in CT_{max} calculated for a species using distinct methods have led to debate over which method(s) are the most ecologically relevant (Terblanche *et al*., 2011; Rezende *et al*. 2014). Our results suggest that multiple methods can provide insight into different aspects of a species' life-history. With respect to the slow-ramp and fast-ramp methods used here, we found evidence that slow-ramp CT_{max} was correlated with nest-specific traits of a species (e.g., thermal dependence of brood), while fast-ramp was not (Fig. 12). In contrast, fast-ramp CT_{max} showed strong signatures of selection among populations of the *A. rudis* complex across a latitudinal cline, while slow-ramp CT_{max} did not. Therefore, both measurements of CT_{max} are ecologically relevant, but they are relevant in unique contexts.

Here, fast- and slow-ramp CT_{max} varied among species (Fig. 11), suggesting that different life histories could lead to differences in thermal adaptive responses. This difference among species is particularly large for species in the *A. rudis* complex, which have a higher relative CT_{max} with the fast-ramp compared to the slow-ramp. Species in the genus *Aphaenogaster* are considered to be more cold-

adapted than some of the more thermophilic co-occurring species, such as *Crematogaster lineolata*. Such differences among species might provide insight into how these species will respond to climatic warming. For instance, if a species like *C. lineolata* can deal with longer periods of high temperatures by slowly acclimating to achieve a higher CT_{max}, selection might favor this response if mean temperatures steadily increase. Mean increases and increased duration of higher temperatures may lead to increased activity of thermophilic species that can outcompete species that forage at a lower or more narrow temperature ranges. This in turn, could lead to shifts in abundance and community dynamics (Chick *et al*., *in review*; Warren and Chick, 2013). Alternatively, if over the long-term there is an increase in mean temperatures as well as an increase in the number of extreme heating events, species that are unable to forage in warmer conditions for longer periods of time might need to seek refuge inside the nest, leading to decreases in foraging time and subsequently, decreases in colony size and growth (Penick *et al.*, *in review*).

A species' thermal performance does not depend only on CT_{max} but also on other thermal dependent traits that vary among species. In ants, we found that differences in development rate at cool temperatures can also mediate how different species are affected by temperature change (Penick *et al., in prep*). Slow-ramp CT_{max} correlated with thermal requirements for pupal development, a nest specific trait, while fast-ramp CT_{max} did not (Fig. 12), suggesting a connection between slow-ramp CT_{max} and the nest environment, whereas fast-ramp CT_{max} may be more tightly linked to foraging, as social insect workers experience a different thermal environment when they leave the nest to forage. In this case, individuals may experience more dramatic and faster changes in temperature than they did when they were buffered inside the nest. Here it may benefit a worker to be able to withstand abrupt changes in temperature that also correlate with latitude. We find evidence that fast-ramp CT_{max} is under stronger

selection across a latitudinal temperature cline, while slow-ramp stays constant. This makes sense, as species that are able to thermoregulate may maintain relatively similar nest temperatures in regions where outside temperature varies to a much greater degree. In these cases there would be little selection on slow-ramp CT_{max}, but fast-ramp CT_{max} may need to increase in regions where workers face higher temperatures outside the nest. Previous research with some of these same species showed that cool, rather than warm, temperatures are the constraining factors of brood production and colony growth by slowing development and shortening the length of the growing season (Penick *et al*., *in review*). Since ground-nesting species can avoid excessive heat and optimize brood development by moving deeper into the soil column to find a thermal optimum, dealing with high temperatures and rapid increases in temperatures may not have a negative impact on the development of brood, even in cold-adapted species. Colony size and growth may actually be more constrained by the high temperatures outside the nest, as they may decrease foraging times and limit resource acquisition, especially in cold-adapted species. In a related study, we used active warming chambers (see Pelini *et al.* 2011a for experimental design) to monitor 24-hour activity cycles and found that with only 1°C of warming, *Aphaenogaster spp.* decreased its foraging time by approximately 5 hours (from 7 to 2 hrs) while *Crematogaster lineolata* increased its activity time threefold (from 8 hours of foraging to 24 hrs (Fig. 13)). Since behavioral thermoregulation can be a coping mechanism to allow the colony to have the highest development possible, there may be tradeoffs of optimal temperatures for development and optimal temperatures for foraging.

Many of these same patterns exist within species as well. When examined within-species variation in CT_{max} in the genus *Aphaenogaster* in the eastern US, we found that fast-ramp CT_{max} is correlated with mean annual temperature (MAT) of source population (r^2=0.74, P<0.001; Fig. 14a), but slow-ramp CT_{max} is not

related to MAT (r^2=0.02, P=0.28; Fig. 14b). These differential responses suggest that selection may act on these thermal traits independently. The underlying coping mechanisms responsible for the fast-ramp CT_{max} have been under selection among populations while mechanisms involved with slow-ramp CT_{max} appear to remain stable within *Aphaenogaster* ants. One explanation for this is that thermoregulatory behaviors inside the nest may buffer environmental selection on slow-ramp CT_{max}, but fast-ramp CT_{max} changes among populations because these populations experience a larger variation in mean annual temperatures.

Differences in thermal limits calculated using distinct ramping speeds suggests the responses we measured depend on different physiological mechanisms. For example, heat shock proteins may contribute to the response at slow ramping speeds, where genes require more than 10 minutes to begin upregulation. In contrast, the short duration of fast-ramp trials may reach CT_{max} before heat shock proteins are upregulated. So, heat shock proteins may contribute to the response of an organism at slow ramping speeds, but not fat-ramping speeds. For each of the 14 ant species we examined, fast- and slow-ramp methods yielded different CT_{max} values based on ramp rate that are only loosely correlated (Fig. 10). An organism's ability to tolerate warming might result from selective forces acting on physiological processes to produce adaptive coping mechanisms. If this is the case, we might expect this to produce differences among populations as well as species.

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APPENDIX III

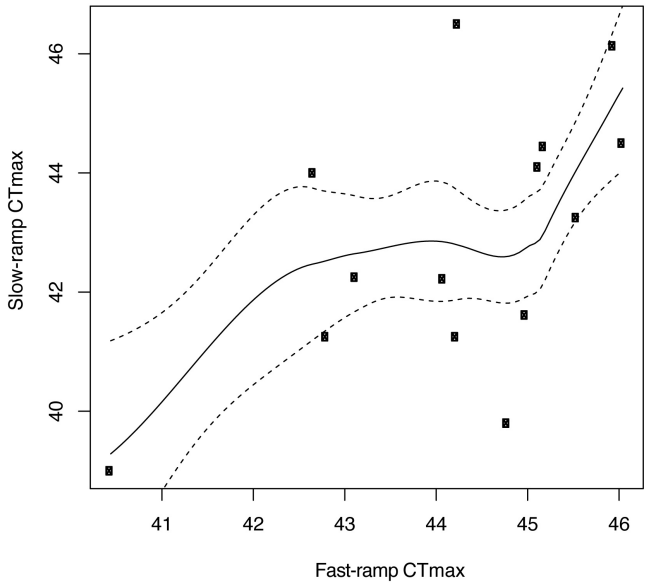

Figure 10. Fast- and slow-ramp methods yield different CT_{max} values for each species (points) that are only loosely correlated.

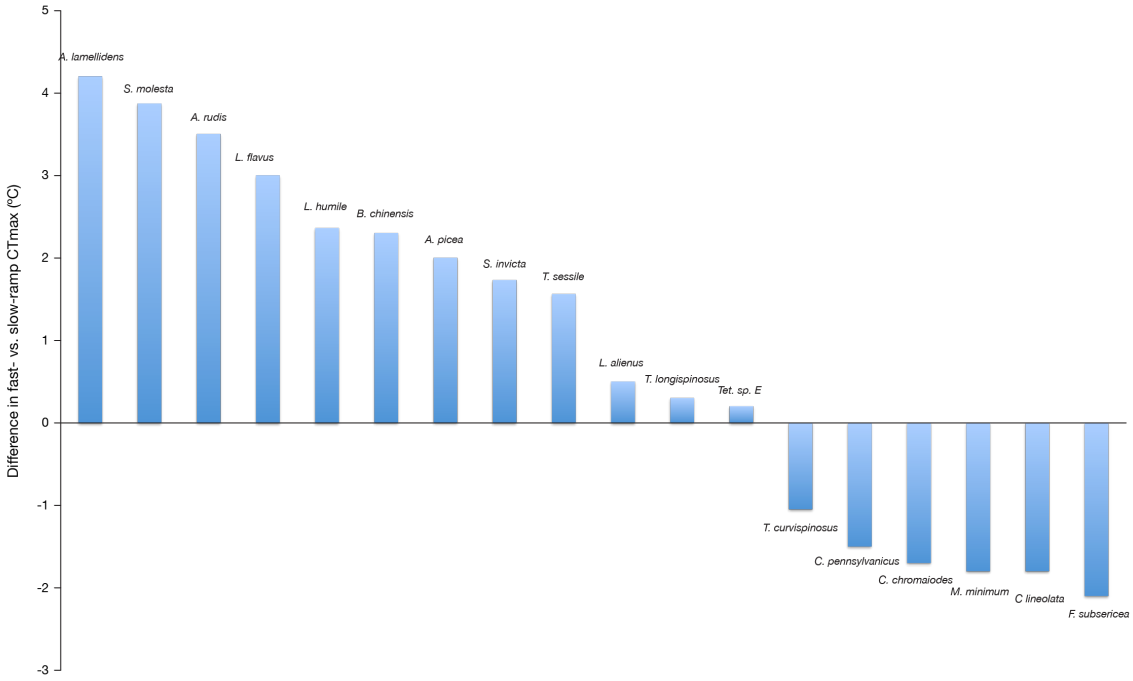

Figure 11. The difference between fast-ramp vs. slow-ramp CT_{max} varies among species. The difference is particularly large for *Aphaenogaster* spp., which have a higher relative CT_{max} with the fast ramp compared to the slow.

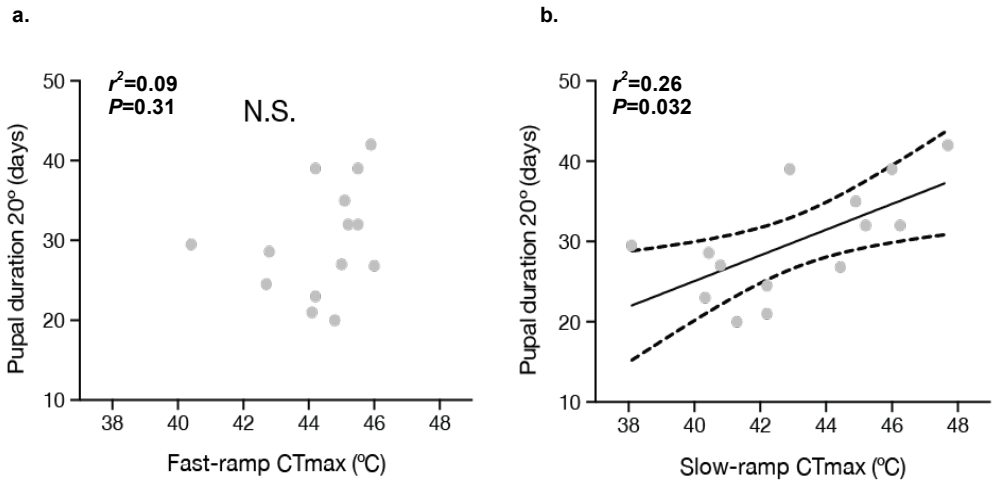

Figure 12. (a) Fast-ramp CT_{max} did not correlate with thermal requirements for pupal development (r^2=0.09, P=0.31), a nest specific trait, while (b) slow-ramp CT_{max} did (r^2=0.26, P=0.032). This suggests a connection between slow-ramp CT_{max} and the nest environment, whereas fast-ramp CT_{max} may be more associated with foraging**.**

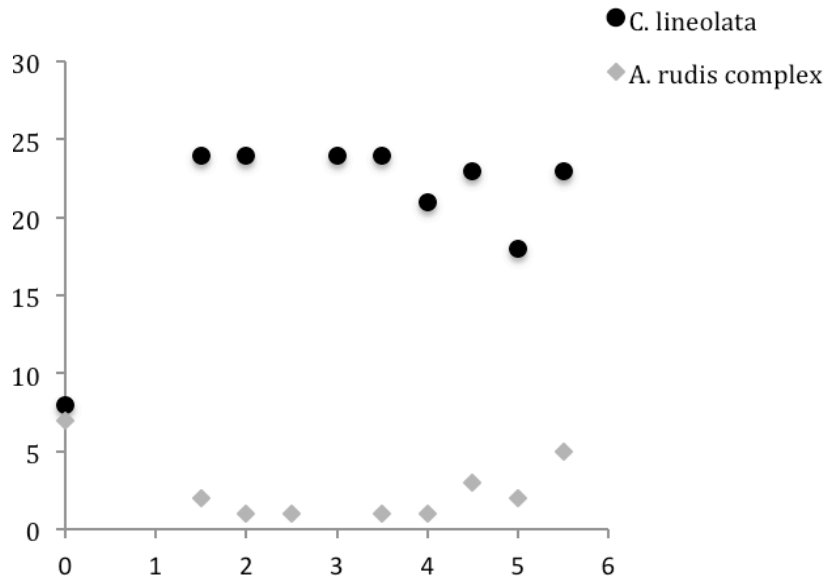

Figure 13. Foraging time decreased by 5 hours with 1°C of warming in *Aphaenogaster spp*. but increased threefold in *Crematogaster lineolata*, increasing from 8 hours to 24 hours. Changes in foraging duration with warming may lead to cascading effects of decreased colony size, growth rate, or abundance.

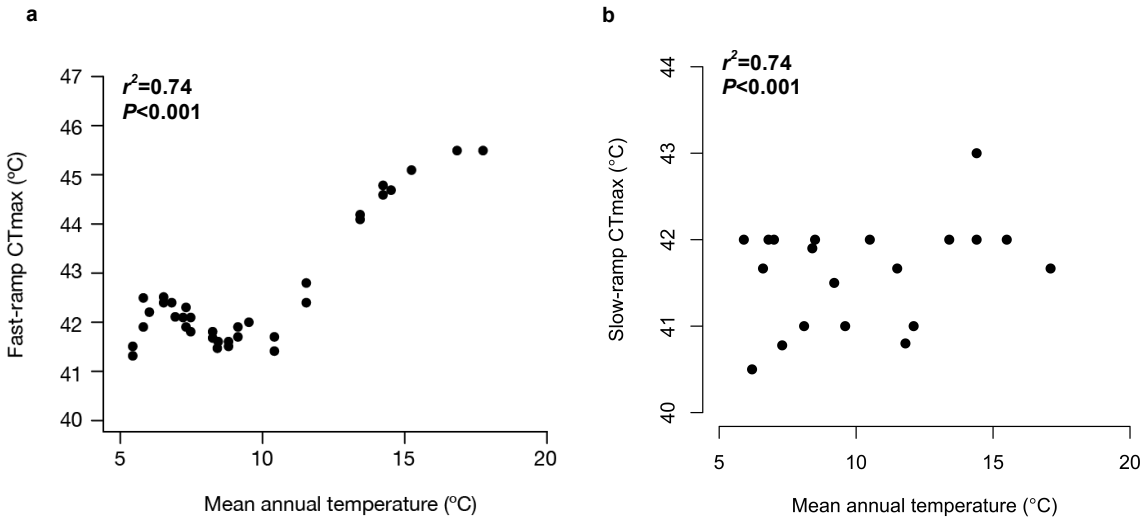

Figure 14. Within *Aphaenogaster* ants, (a) fast-ramp CT_{max} was correlated with mean annual temperature of source population (r^2=0.74, P<0.001), but (b) slow-ramp CTmax showed no relationship among populations (r^2=0.02, P=0.28). This suggests fast-ramp CT_{max} has been under selection among populations while slow-ramp CT_{max} appears to remain stable within the *A. rudis* complex.

CONCLUSION

My dissertation examined the thermal physiology of ant species in deciduous forests in the eastern U.S. to address the overarching question: *how does temperature influence diversity?* I addressed this broad ecological question by first examining physiological limitations on individual ant colonies, and then by scaling up to examine both inter- and intra-specific variation across geographic gradients of elevation and latitude.

Through experimental manipulations, I found that temperature was an important factor governing the abundance, density, and distributions of ants, yet, while temperature was an important abiotic filter for the regional species pool, it was not the only constraint on diversity. Through null model analyses and observational data, I determined that biotic interactions also helped shape the distributions and diversity of ants and, more interesting, that the drivers of diversity were dependent on geography. Many studies have speculated why there are more species in some areas than in others, yet this is one of the first studies to mechanistically show that diversity and distributions are geographically-dependent. This finding is important if we are to understand not only how communities are structured, but also, how that structure might be disrupted in the face of a changing climate.

When considering the effects of climate warming on ant communities, the extent to which populations can adapt to local environments will prove an important factor for predicting responses. For a portion of my dissertation, I conducted a common garden experiment where I, along with collaborators at North Carolina State University, collected ant colonies from an extensive latitudinal gradient of 16°N and reared them in the lab under different temperature treatments to determine if thermal traits could be a result of local adaptation or acclimation. We

found that thermal physiological limits did not change with rearing temperature, but rather mirrored the physiological results of ants collected from the field. Results from this experiment indicate that populations of the genus *Aphaenogaster* are likely adapted to their local thermal regimes and that physiological traits may be genetically constrained. Building on the common garden experiment, I asked if an organism's ability to tolerate warming might result from selective forces acting on physiological processes to produce adaptive coping mechanisms? To address this concern, I examined different warming speeds (fast and slow) to see if responses associated with heat tolerance differed between species, as well as among life stages within a species. We found that the slow thermal response correlated with traits associated with the nest environment of species and varied little among populations within a species. In contrast, the fast thermal response did not correlate with nest-based traits, but showed strong signatures of selection along a climate gradient. These results suggest that different life stages of organisms might cope with climate change in different ways.

While there is still research to be done to determine the mechanisms mediating coexistence and how communities might respond to climate warming, this dissertation begins to address this issue in a mechanistic manner. My results will aide in our understanding of how communities are structured along gradients and how variation among individuals, populations, and communities, might scale up to influence the distributions of species now, and in the future.

VITA

Lacy Danikas was born in Myrtle Beach, SC. She graduated from Socastee High School in May 2002 and went on to pursue a Bachelor's Degree in Biology at Francis Marion University from 2002-2006. While taking science courses at FMU, her love of biology grew and she traveled to exotic places learning about natural history. While at FMU she met Brendan Chick and the two were married while Lacy was earning her Master's Degree in Zoology from Middle Tennessee State University. After graduating from MTSU, Lacy came to the University of Tennessee in August 2010 to begin her doctoral degree researching ants in the Great Smoky Mountains.