A physiological trait-based approach to predicting the responses of species to experimental climatic warming

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Running title: Predicting ant responses to warming
Abstract

Physiological tolerance of environmental conditions can influence species-level responses to climatic change. Here, we used species-specific thermal tolerances to predict the community responses of ant species to experimental forest-floor warming at the northern and southern boundaries of temperate hardwood forests in eastern North America. We then compared the predictive ability of thermal tolerance versus correlative species distribution models (SDMs) which are popular forecasting tools for modeling the effects of climatic change. Thermal tolerances predicted the responses of 19 ant species to experimental climatic warming at the southern site, where environmental conditions are relatively close to the ants’ upper thermal limits. In contrast, thermal tolerances did not predict the responses of the 6 species in the northern site, where environmental conditions are relatively far from the ants’ upper thermal limits. Correlative SDMs were not predictive at either site. Our results suggest that, in environments close to a species’ physiological limits, physiological trait-based measurements can successfully forecast the responses of species to future conditions. Although correlative SDMs may predict large-scale responses, such models may not be accurate for predicting site-level responses.

Keywords: critical thermal maximum, global change, Formicidae, physiology, species distribution model, thermal tolerance

Introduction

Predicting biological responses to climatic change is critical (Araújo et al. 2005), but a number of researchers have begun to emphasize the potential unpredictability of species’ responses to climatic change (e.g., Hill et al. 2002, McGeoch et al. 2006, Pelini et al. 2009, Doak and Morris
If species-specific traits covary with their responses to climatic change, such traits can be used to predict community change (Diamond et al. 2011, Angert et al. 2011). Physiological traits have been especially successful in predicting responses of individual species to climatic change (Chown et al. 2004, Helmuth et al. 2005, Buckley 2008, Deutsch et al. 2008, Pörtner and Farrell 2008, Huey et al. 2009, Kearney and Porter 2009, Sinervo et al. 2010, Diamond et al. 2012). However, these predictions have only been evaluated through simple correlations with historical, current, or projected future conditions (reviewed in Rowland et al. 2011). Experimental manipulations provide a unique, but relatively under-used approach for evaluating the degree to which physiological traits may inform the responses of species to climatic change.

Here, we used results from a pair of large-scale experimental climatic warming arrays, positioned near the northern (Harvard Forest; Petersham, Massachusetts; ≈42° N lat.) and southern (Duke Forest; Hillsborough, North Carolina, USA; ≈36° N lat.) boundaries of temperate hardwood forests in eastern North America to test the ability of physiological thermal tolerance to predict responses of ant species to warming. In the extensive literature on ecological effects of global climate change, such experiments are rare because they are expensive and time-consuming. Temperature-induced changes in community composition (Walker et al. 2006), nutrient cycling (Rustad et al. 2001), and phenology (Wolkovich et al. 2012) have been previously documented in such experimental warming arrays, although ours is the first study to incorporate independent measures of physiological tolerance. We manipulated temperatures among experimental open-top chambers in a regression design that boosted air temperature in each chamber from 1.5 to 5.5 °C above ambient. This range of temperatures encompasses a variety of future warming scenarios (IPCC 2007), and induced a wide range of species-specific responses in ant activity density. The key question we address here is what is the best predictor
of changes in ant activity density in the experimental chambers: measured physiological
tolerances of individual species or the species-specific predictions of MaxEnt, a popular species
distribution model (SDM; reviewed in Elith and Leathwick 2009)?

Although SDMs are typically used to predict distributions at large spatial scales, effects
of the changing climate on species geographic ranges ultimately reflects population dynamics
and the activity of individuals at local scales. By comparing 3 independent sources of data
(activity responses to warming in a climatic change field experiment, measurements of
physiological tolerance of individual species, and MaxEnt predictions) at two locations (Harvard
Forest and Duke Forest), we have a unique chance to evaluate MaxEnt predictions.

Ants are a good choice for this kind of comparison because they are ecologically
important thermophiles in eastern deciduous forests (Ellison et al. 2012), appear commonly in
the warming chambers at both sites, and their geographic ranges are relatively well known
(Fitzpatrick et al. 2011). For each of the ant species recorded in the experimental chambers, we
independently measured their thermal tolerance (critical thermal maximum, CT_{max}) and
quantified their projected changes in probability of occurrence under several climatic change
scenarios using correlative SDMs based on thermal indices of the environment.

We predicted that: (1) species with higher thermal tolerances would increase in
abundance with experimental warming, owing to the widespread pattern among ectotherms of
positive correlations between CT_{max} and the temperature at which optimal performance is
reached (T_{opt}) (Huey and Kingsolver 1993), (2) species with greater probabilities of occurrence
under projected climatic warming according to correlative SDMs would become more abundant
as experimental temperatures increased, and (3) CT_{max} would be a better predictor of responses
to warming for ants at the southern forest boundary (Duke Forest) than at the northern forest
boundary (Harvard Forest). This final prediction is based on recent studies suggesting that ectothermic species at lower latitudes are relatively more sensitive to changes in temperature because of their narrow thermal performance curves, and because environmental temperatures are relatively closer to their upper thermal limits. By comparison, species at higher latitudes tend to be more tolerant of changes in temperature because of their broader thermal performance curves and because environmental temperatures at high latitudes are relatively far below their upper thermal limits (Appendix A; see especially Fig. 1 in Tewksbury et al. 2008; see also Deutsch et al. 2008, Dillon et al. 2010). In general, performance begins to decline sharply when $T_{\text{opt}}$ is exceeded, which imposes strong limitations on occupying thermal environments that overlap the range of temperatures between $T_{\text{opt}}$ and CT$_{\text{max}}$.

Materials and Methods

**Warming chambers and Ant collections.** Both the Harvard Forest and Duke Forest sites include 12 open-top experimental plots (5 m in diameter, and raised approximately 5 cm off of the ground to allow ants to move unrestricted) in the forest understory (details in Pelini et al. 2011). Nine chambers are heated (by the addition of warmed air) according to a regression design of 0.5 °C increasing intervals from 1.5 to 5.5 °C above ambient air temperature (hereafter referred to as $\Delta_c$), and three chambers are unheated controls ($\Delta_c = 0$). We used pitfall sampling to estimate ant activity density (Appendix B): monthly pitfall samples were conducted at Duke and Harvard Forest (April 2010 - September 2011).

**Thermal tolerance and Species distribution models.** We defined the critical thermal maximum (CT$_{\text{max}}$) as the temperature at which muscle coordination was lost (Lutterschmidt and Hutchison 1997), an ecologically relevant measure of CT$_{\text{max}}$ as the temperature at which an
individual could not escape to a non-lethal thermal environment (Lighton and Turner 2004). Ant workers of different species were collected in the forest adjacent to the chambers, and their thermal tolerances were tested individually (minimum 8 individuals per species at each site) in a heat block that generated a 2 °C temperature increase every 10 minutes starting at 36 °C. At the end of every 10 minute interval, individual ants were checked for the loss of muscular coordination (Appendix B).

For species distribution models (SDMs), current climatic data were obtained from WorldClim (Hijmans et al. 2005), and projected future climatic data (for the year 2080 based on the CCCMA-CGCM2 model) from the International Centre for Tropical Agriculture (CIAT) (Ramirez and Jarvis 2008; Appendix B,C,D,E). North American occurrence data (presence-only) for each of the ant species present in the pitfall traps at Duke and Harvard Forests were obtained from the primary literature and museum records (Fitzpatrick et al. 2011).

**Analyses.** We collected 24 and 11 species in pitfall traps at Duke and Harvard Forest respectively (excluding the non-ground foraging ant species *N. texanus* and *C. obliquus*; Appendix B). Of these species, we were able to obtain corresponding physiological and distribution data for 19 and 6 species, respectively. Average CT$_{\text{max}}$ values were calculated for each species and used as a predictor variable in regression models of ant activity density responses in the experimental chambers. All analyses were performed in R (version 2.13.1; R Development Core Team 2011).

**Physiological models.** We used ANOVA to test whether physiological tolerance to high temperatures influences ant abundance (effectively, worker activity density, given comparable sampling areas in our study; Longino and Colwell 2011) in response to experimentally simulated climatic warming. Cumulative worker density across sampling events was considered the
response variable, and $CT_{\text{max}}$, $\Delta c$, and the interaction of $CT_{\text{max}}$ with $\Delta c$, were considered as continuous fixed-effect predictor variables. All assumptions of ANOVA were met (see below).

**MaxEnt models.** We fit maximum entropy (MaxEnt) correlative species distribution models (SDMs) for each species with standard settings for the *maxent* function from the *dismo* package in R (Hijmans et al. 2011). Three sets of MaxEnt models were developed based on current and future (2080) environmental variables most relevant to manipulated aspects of the experimental arrays (i.e., thermal indices): 1) mean annual temperature, 2) mean temperature during the warmest annual quarter, and 3) maximum temperature during the warmest annual quarter. We used these thermal indices to develop models to predict the probability of occurrence within North America, and then extracted the probability of occurrence values for each species at each site under current and future climates. Typically, projected changes in probability of occurrence across a species’ entire range are used to infer species’ responses to climatic change (Fitzpatrick et al. 2008). Here, we restricted our consideration of MaxEnt-derived changes in probability of occurrence to the approximately 1 km$^2$ areas containing the Duke and Harvard Forest experimental warming sites. In this way, the spatial scales were comparable for comparisons of thermal tolerances, MaxEnt predictions, and responses to experimental warming. MaxEnt usually performs more poorly when it is underparameterized than it does when it is overparameterized (Warren and Seifert 2011); to address this issue, we used expanded sets of MaxEnt models fit with all 19 bioclim variables (Appendix B,C). These results were qualitatively similar to the thermal index-only models. Therefore, we present the MaxEnt models based on just the thermal indices (Hijmans and Graham 2006).

**Model Comparisons.** We used ANOVA to test the ability of physiological thermal tolerance and correlative SDMs to predict the responses of ants to experimentally simulated
climatic warming. The slope of the linear relationship between ln(cumulative worker density across all sampling events) and $\Delta_c$ was considered the response (Appendix B,F), and $CT_{\text{max}}$ and the difference in the probability of occurrence of a particular ant species based on current and future (2080) climate derived from MaxEnt models (future – current, such that positive values indicate increased probability of occurrence under climatic warming) were considered continuous fixed effects. The calculation of the thermal accumulation slope was not possible for a small fraction (< 1%) of ant species which only occurred within a single chamber across all sampling events (Appendix B). Therefore, we also examined a complementary response variable, the maximal accumulation temperature (positively correlated with thermal accumulation slope; $r = 0.78$), which allowed us to include these species in our analyses. The maximal accumulation temperature was defined as the mean of the chamber deltas ($\Delta_c$) in which a given species occurred, where the contribution of each $\Delta_c$ was weighted by cumulative worker density (across all sampling events) for that given species in that given chamber. Cumulative worker densities were normalized to sum to one (for a given species among all the chambers in which it occurred) prior to this calculation.

For simplicity, hereafter we explicitly use “$CT_{\text{max}}$” to refer to the critical thermal maximum, “$\Delta_c$” to refer to the degrees Celsius above ambient for each experimental warming chamber, and “MaxEnt prediction” to refer to the change in probability of occurrence between current and future climates; similarly, we refer to the response variables as “thermal accumulation slope” (slope of the linear relationship between ln(cumulative worker density) and $\Delta_c$) and “maximal accumulation temperature” (mean $\Delta_c$ weighted by cumulative worker density).

In all of these analyses, it is the different species, not the experimental chamber or the site, that represent the replicate observations.
Phylogenetic autocorrelation. To account for the potential influence of phylogenetic autocorrelation on our results, we re-ran our models of ant responses to warming using phylogenetic generalized least squares (PGLS from the CAIC package; Orme et al. 2009) under an assumption of trait evolution by Brownian motion. For each model, the maximum likelihood estimate of $\lambda$ was used to scale the model covariance (Appendix B,G).

Results and Discussion

Predictive ability of thermal tolerance. At the low-latitude site (Duke Forest), responses of ant species to experimental warming (1.5 to 5.5 °C above ambient temperature) were well-predicted by physiological tolerance of the ants to high temperatures (critical thermal maximum, $CT_{\text{max}}$). ANOVA revealed a significant interaction effect between $CT_{\text{max}}$ and $\Delta c$ on post-treatment cumulative worker density ($F_{1,174} = 6.33, P = 0.0128$; the main effects of $CT_{\text{max}}$: $F_{1,174} = 0.491, P = 0.485$, and $\Delta c$: $F_{1,174} = 0.290, P = 0.591$, were not significant), indicating the relationship between worker density and the degree of experimental warming was contingent upon the ants’ thermal tolerance. Specifically, species with higher thermal tolerance had greater worker densities under warmer conditions (Fig. 1A). In contrast, at the high latitude site (Harvard Forest), responses of ants to experimental warming were poorly predicted by individual $CT_{\text{max}}$ (Fig. 1C). ANOVA revealed non-significant effects of $CT_{\text{max}}$ ($F_{1,43} = 0.127, P = 0.723$, $\Delta c$: $F_{1,43} = 1.51, P = 0.226$, and their interaction: $F_{1,43} = 1.40, P = 0.243$). Instead, worker densities were greatest in the warmest experimental treatments: regardless of $CT_{\text{max}}$, all 6 species achieved their maximum densities in warming treatments of 3.5 °C above ambient or greater (Appendix H). At the high latitude site, maximum daily temperatures never exceeded 38 °C (the lowest $CT_{\text{max}}$ of species at Harvard Forest) in any of the warming chambers. As a consequence, there
was little risk of any species exceeding its CT_{max}, and ant performance may improve under the
warmest treatments as ants approach their T_{opt}. However, at the low-latitude site, maximum daily
temperatures exceeded 37 °C (the lowest CT_{max} of species at Duke Forest) during 9% of the year
(based on mean hourly temperatures) among all of the warming chambers. As a consequence,
some species are likely to have experienced temperatures in excess of their CT_{max} in the warmest
treatments, resulting in the differential representation of worker densities among species in the
warming treatments.

Collectively, these results suggest that CT_{max} may be a useful predictor of species’
responses to climatic warming in regions with relatively warm baseline temperatures where
species are close to their upper thermal limits. CT_{max} may not be a good predictor in regions with
relatively cool baseline temperatures where species are far from their upper thermal limits

Predictive ability of correlative species distribution models. The MaxEnt models based
on mean annual temperature, mean temperature during the warmest quarter, and maximum
temperature during the warmest quarter for current and future (2080) climates were themselves
statistically well supported: species occurrences were significantly correlated with these thermal
variables, and AUC_{test} values (based on current climatic conditions) were > 0.8 in all cases (to
obtain AUC_{test} values, 20% of the data were withheld for testing using k-fold partitioning). We
emphasize, however, that our primary interest was in relative differences among species in the
change in probability of occurrence from current to future conditions, and how these differences
potentially relate to species’ responses to experimental warming, rather than in the precision of
individual SDMs.
In this respect, correlative SDMs were poor predictors compared with CT\textsubscript{max} at the southern site, and equally poor predictors as CT\textsubscript{max} at the northern site (Fig. 1B,D; Appendix C,D,E). ANOVAs of thermal accumulation slopes revealed significant effects of CT\textsubscript{max}, but non-significant effects of MaxEnt predictions (calibrated with mean temperature during the warmest quarter) at the southern site: CT\textsubscript{max}: $F_{1,14} = 10.3$, $P = 0.00639$, MaxEnt: $F_{1,14} = 0.560$, $P = 0.467$. ANOVAs of thermal accumulation slopes revealed non-significant effects of both CT\textsubscript{max} and MaxEnt predictions (calibrated with mean temperature during the warmest quarter) at the northern site: CT\textsubscript{max} ($F_{1,3} = 0.159$, $P = 0.717$, MaxEnt: $F_{1,3} = 1.84$, $P = 0.268$). Results for ANOVAs of maximal accumulation temperature were qualitatively similar (Appendix I). These results do not reflect our particular choices of thermal index or future climate models, and were robust to many alternative calibrations of the MaxEnt models (Appendix C,E).

Correlative SDMs offer many advantages for ecologists: they are easy to develop and can successfully predict range shifts in some species (Kearney et al. 2010). The relative ease of developing correlative SDMs results in part from the simplification of the biological world inherent in their use (Fitzpatrick et al. 2007). The application of correlative SDMs in climatic change impact assessment has been criticized (Dormann 2007, Fitzpatrick and Hargrove 2009), largely on the basis that correlative SDMs ignore evolution and complex interactions between species, which may themselves change as the climate changes (Schmitz et al. 2003). We are careful here to note that our correlative SDMs based on environmental thermal indices are relatively simplistic, and that more sophisticated methods for generating species distribution models can be applied when more detailed data are available. For example, SDMs have incorporated additional variables such as land use (Heikkinen et al. 2006), and mechanistic versions of SDMs are capable of incorporating effects of physiology and demography (Buckley
2008, Kearney and Porter 2009). However, such methods trade off predictive power with greater investment in data collection and analysis. Although more sophisticated modeling techniques are always possible, the results of our study suggest physiological traits alone can be important predictors of responses of individual species to climatic warming in regions where species are close to their physiological limits. In such cases, physiological-based models outperform relatively simple forms of correlative SDMs, at least with respect to experimental climatic warming at the site level. Perhaps SDMs perform better only at the large spatial scales at which they are typically used (Heikkinen et al. 2006). On the other hand, if they are to be of practical use, they should have some relevance to changes at individual sites. The fact that simple laboratory measures of thermal tolerance (CT$_{\text{max}}$) are good predictors of activity density responses in experimental warming arrays suggests that additional measurements of behavioral and physiological responses to warming may be more productive than continued refinements of correlative SDMs.

*What else is needed for improved predictive ability?* Depending on the metric used to quantify responses to warming, thermal tolerance (CT$_{\text{max}}$) alone explained a sizable fraction of the variation (38 to 42%) among species at the warm site. Although indirect responses (including indirect species effects and interactions mediated by temperature) may play an important role, direct effects of temperature on performance are critical for understanding the responses of ants, and probably many other ectotherms, to global warming. The unexplained variation in our analyses can be partly understood by focusing on the biology of the outlier species. For example, at warm site, *Camponotus americanus* and *C. pennsylvanicus* tended to occupy relatively cool chambers despite their intermediate CT$_{\text{max}}$ values; at a global scale, such forest specialist species tend to be relatively intolerant of warming (Diamond et al. 2012). In addition, two other
Camponotus species (C. chromaiodes and C. castaneus), tended to occupy moderately heated
chambers—chambers below or at the level predicted by the regression of ant responses to
warming against CT_{max}. Such phylogenetic clustering suggests the possible presence of shared
developmental or genetic constraints on thermal tolerance. We did indeed detect non-zero levels
of phylogenetic signal in the model, but CT_{max} was still a significant predictor of responses to
warming at the low latitude site (Appendix G).

Our results suggest that the subset of the species in the regional species pool in the
southeastern United States that will become more abundant with climatic warming will be those
with high thermal tolerances. Although our study focused on those species already present at the
study sites the same trends might also hold more generally within the larger regional species
pool. We speculate that species with high thermal tolerances from distant southern sites might be
among the first to colonize the new climatic environments generated by regional warming.

Similarly, if one considers the global species pool of ants being transported introduced around
the world (e.g., Suarez et al. 2005), those with high thermal tolerances are good candidates for
successful establishment in novel environments that have experienced warming.

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Literature Cited


International Centre for Tropical Agriculture CIAT.

<http://gisweb.ciat.cgiar.org/GCMPage>.


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Supplemental Material

Appendix A. Relationships between environmental temperature, warming chamber temperature manipulations and hypothesized ant thermal performance curves at the high latitude (Harvard Forest) and low latitude (Duke Forest) sites.

Appendix B. Methodological and analytical details on the construction and evaluation of models of ant responses to climate warming.

Appendix C. Model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions developed with alternative GCMs.


Appendix E. Model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions developed with alternative thermal indices.

Appendix F. Sample calculations of thermal accumulation slope.

Appendix G. Phylogenetic model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions.

Appendix H. Ant worker density as a function of warming treatment at Harvard Forest.

Appendix I. Regressions of maximal accumulation temperature as functions of thermal tolerance and MaxEnt predictions.

Appendix J. Regressions of thermal accumulation slope (including standard errors) as functions of thermal tolerance and MaxEnt predictions.
Figure legends

Figure 1. The predictive ability of thermal tolerance versus species distribution models in ant responses to warming at high and low latitudes: thermal accumulation slope (the slope, $\beta$, of the linear relationship between ln(cumulative worker density) and chamber delta ($\Delta_c$, °C)) as a function of (A,C) the critical thermal maximum ($CT_{\text{max}}$, °C), and (B,D) MaxEnt prediction (the change in probability of occurrence across MaxEnt models based on current and future (2080) climate as defined by mean annual temperature) at (A,B) the low latitude site (Duke Forest), and (C,D) the high latitude site (Harvard Forest). Each point represents a single species; solid orange lines represent simple linear regressions (p-values indicate whether the slope is significantly different from zero), and dashed blue lines represent 95% confidence intervals.

APPENDIX A. Relationships between environmental temperature, warming chamber temperature manipulations and hypothesized ant thermal performance curves at the high latitude (Harvard Forest) and low latitude (Duke Forest) sites.
FIG. A1. Relationships between environmental temperature, warming chamber temperature manipulations and hypothesized ant thermal performance curves at the high latitude (Harvard Forest) and low latitude (Duke Forest) sites. The left panel depicts the
current temperature of the warmest annual quarter (°C) derived from WorldClim. The two rightmost panels depict hypothesized thermal performance curves (blue lines), with relative performance as a function of temperature at the high latitude (top panel) and low latitude (bottom panel) sites. The color gradients correspond with the current temperature of the warmest annual quarter (ambient temperature) at each site, and temperatures of the warmest annual quarter after applying the warming chamber treatments (1.5 to 5.5 °C above ambient temperature). Note that environmental temperatures in the warming chambers are much closer to the thermal optimum (\(T_{\text{opt}}\)) and critical thermal maximum (\(CT_{\text{max}}\)) at the low latitude site compared with the high latitude site.
Ecological Archives A/E/M000-000-A1


APPENDIX B. Methodological and analytical details on the construction and evaluation of models of ant responses to climate warming.

Methodological details

Pitfall collections
To sample ants, we placed four pitfall traps (90 mL volume) containing propylene glycol (30 mL; Prestone, LowTox) flush with the soil surface in each chamber. During each sampling event, traps were left out for a 48-hour sampling period (performed monthly; see below). At the end of the 48-hour sampling period, individual ants recovered in the pitfall traps were removed from the propylene glycol and preserved in 95% ethanol. All ants were identified to the species level; pinned voucher specimens are retained at North Carolina State University, and at Harvard Forest.

Monthly pitfall samples were conducted at Duke and Harvard Forest (April 2010 - September 2011). Pitfall data also were collected for each chamber following chamber construction, but prior to the setting of experimental temperature treatments (September - November 2009). We examined such ‘pre-treatment’ data for potential preexisting biases in species abundance across chambers. A gap exists between the pre- and post-treatment data because we restrict our analyses of post-treatment data to those data collected after the stabilization of Δc_s in experimental chambers which required approximately 4 months. We restricted our analyses to those ground-foraging ant species which were sampled in the pitfall traps at Duke and Harvard Forests, and excluded data on a primarily subterranean, exceptionally rare species that does not nest in the chambers (Neivamyrmex texanus), and a canopy specialist species (Camponotus obliquus).

Thermal tolerance
Colony fragments of ants (workers only) were collected from open and forested areas adjacent to the Duke and Harvard Forest warming sites, and comparable habitats within Wake Co. (North Carolina, USA) and Worcester Co. (Massachusetts, USA). Colony fragments were maintained with continuous access to food and water at a non-stressful temperature of 25 °C, ensuring ants were in good condition prior to thermal testing (testing occurred within 24 hours of collection). Ants were placed individually into 1.5mL Eppendorf tubes which contained cotton in the lid cap to eliminate a potential thermal refuge. The tubes were transferred to a heating dry block (Thermal Lok USA Scientific), and the temperature was increased by 2 °C every 10 minutes starting at 36 °C until the loss of ant muscular coordination which indicated CT_{max} was reached.

Species distribution models
Current climatic data were obtained from WorldClim at a 30 arc-second (1 km) resolution (Hijmans et al. 2005). Statistically downscaled global climate change models (GCM) based on the third IPCC Assessment Report were obtained from the International Centre for Tropical Agriculture (CIAT) (Ramirez and Jarvis 2008), and used to derive predicted future climate data for 2080. We examined a range of different GCMs (CCCMA-CGCM2, CSIRO-MK2, and HCCPR-HADCM3 at a 30 arc-second resolution); because results were similar across different climate models, we focus on results from the CCCMA-CGCM2 model (Appendix C,D). This model predicts a 4.6 °C increase in temperature at Duke Forest, and 4.8 °C increase at Harvard Forest by the year 2080.

North American occurrence data (presence-only) for each of the ant species present in the pitfall traps at Duke and Harvard Forests were obtained from the primary literature and museum records (Fitzpatrick et al. 2011). The median number of records was 111 species⁻¹ and ranged from 13 to 471 for the Duke and Harvard Forest species examined in our study.

**Phylogenetic autocorrelation**

We fit phylogenetic generalized least squares (PGLS) models where the degree of phylogenetic autocorrelation (Pagel’s $\lambda$) was simultaneously co-estimated. Lambda is a measure of phylogenetic inertia, or how closely the structure in the model residuals resembles the structure of the phylogeny, with greater values indicating greater phylogenetic structure. Phylogenetic associations among ant genera were based on the phylogeny of Moreau et al. (2006). Unknown relationships among species were interpolated as polytomies.

**Supporting analyses and results**

**Potential for pre-existing patterns in ant activity density**

Prior to chamber deltas being set at Duke and Harvard Forest, we found little evidence of systematic variation in the worker density of ants among different chambers (ANOVA revealed a non-significant effect of chamber on pre-treatment cumulative worker density at Duke Forest: $F_{11,74} = 0.317, P = 0.980$, and at Harvard Forest: $F_{11,8} = 0.581, P = 0.802$), indicating our post-treatment results of CT$_{max}$ being predictive of ant activity density do not simply reflect pre-existing patterns of warming chamber colonization.

**ANOVA models based on thermal accumulation slope**

For ANOVA models in which the slope of the linear relationship between ln(cumulative worker density) and $\Delta c$ was considered the response, and CT$_{max}$ and the difference in MaxEnt probability of occurrence between current and future climate were considered continuous fixed effects, two species (*Amblyopone pallipes* and *Temnothorax pergandei*) from Duke Forest were excluded from this analysis owing to their occurrence in only a single temperature treatment (slopes relating ln(cumulative worker density) and $\Delta c$ could not be estimated).

We additionally performed ANOVAs of thermal accumulation slope as functions of CT$_{max}$ and MaxEnt predictions with the residuals weighted by 1/(SE of the thermal accumulation slope). The results were qualitatively similar to our unweighted analyses. We focus on the unweighted analyses, as weighted analyses introduce some degree of systematic bias in which species that naturally occur at low frequency, but nonetheless respond to warming treatments, are necessarily weighted less than more frequently occurring species with comparable responses to the warming treatments (Appendix J).
LITERATURE CITED


APPENDIX C. Model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions developed with alternative GCMs.

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<td></td>
<td></td>
<td>MaxEnt</td>
<td>0.0196</td>
<td>0.890</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>CT_{max}</td>
<td>10.5</td>
<td>0.00589</td>
</tr>
<tr>
<td></td>
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<td>MaxEnt</td>
<td>3.04</td>
<td>0.103</td>
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<tr>
<td></td>
<td>Harvard Forest</td>
<td>maximal accumulation temperature</td>
<td>CT_{max}</td>
<td>0.0843</td>
<td>0.790</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>MaxEnt</td>
<td>0.342</td>
<td>0.600</td>
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<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>CT_{max}</td>
<td>1.43</td>
<td>0.318</td>
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<tr>
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<td></td>
<td>MaxEnt</td>
<td>0.0367</td>
<td>0.860</td>
</tr>
<tr>
<td>HCCPR-HADCM3</td>
<td>Duke Forest</td>
<td>maximal accumulation temperature</td>
<td>CT_{max}</td>
<td>12.0</td>
<td>0.00316</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MaxEnt</td>
<td>1.69</td>
<td>0.212</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>CT_{max}</td>
<td>12.0</td>
<td>0.00385</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MaxEnt</td>
<td>1.18</td>
<td>0.296</td>
</tr>
<tr>
<td></td>
<td>Harvard Forest</td>
<td>maximal accumulation temperature</td>
<td>CT_{max}</td>
<td>2.09</td>
<td>0.244</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MaxEnt</td>
<td>0.0002</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>CT_{max}</td>
<td>0.0206</td>
<td>0.895</td>
</tr>
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<td></td>
<td></td>
<td>MaxEnt</td>
<td>0.462</td>
<td>0.546</td>
</tr>
</tbody>
</table>
MaxEnt models are constructed using all 19 bioclim variables (L-1 regularization using the default settings was employed) to facilitate overall comparisons among different climate models; similar results were obtained using thermal indices (mean annual temperature, mean temperature during the warmest quarter, and maximum temperature during the warmest quarter) as individual predictors.

(Numerator degrees of freedom, denominator degrees of freedom) for predictors: Duke Forest maximal accumulation temperature = (1, 16); Duke Forest thermal accumulation slope = (1, 14); Harvard Forest maximal accumulation temperature and thermal accumulation slope = (1, 3).

**APPENDIX D.** Thermal indices of current and future climates at Duke and Harvard Forests.

**TABLE D1.** Thermal indices of current and projected future climates based on three climate change models at Duke and Harvard Forests.

<table>
<thead>
<tr>
<th>Site</th>
<th>Thermal index</th>
<th>Temperature (°C; current WorldClim, 2080 CCCMA-CGCM2, CSIRO-MK2, HCCPR-HADCM3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duke Forest</strong></td>
<td>Mean annual temperature</td>
<td>14.5, 19.1, 19.6, 19.4</td>
</tr>
<tr>
<td></td>
<td>Mean temperature warmest quarter</td>
<td>24.1, 29.3, 29.2, 30.8</td>
</tr>
<tr>
<td></td>
<td>Maximum temperature warmest quarter</td>
<td>31.4, 38.8, 36.4, 39.3</td>
</tr>
<tr>
<td><strong>Harvard Forest</strong></td>
<td>Mean annual temperature</td>
<td>7.3, 12.1, 14.2, 12.1</td>
</tr>
<tr>
<td></td>
<td>Mean temperature warmest quarter</td>
<td>18.9, 23.7, 24.8, 25.1</td>
</tr>
<tr>
<td></td>
<td>Maximum temperature warmest quarter</td>
<td>27.1, 31.7, 33.0, 34.1</td>
</tr>
</tbody>
</table>

**APPENDIX E.** Model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions developed with alternative thermal indices.

**TABLE E1.** Model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions developed with alternative thermal indices.

<table>
<thead>
<tr>
<th>MaxEnt calibrating variable</th>
<th>Site</th>
<th>Response</th>
<th>Predictor</th>
<th>$F^*$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{an}$</td>
<td>Duke Forest</td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>8.65</td>
<td>0.00960</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>8.91</td>
<td>0.00983</td>
</tr>
<tr>
<td></td>
<td>Harvard Forest</td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>0.0188</td>
<td>0.900</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>1.23</td>
<td>0.348</td>
</tr>
<tr>
<td>$T_{qt}$</td>
<td>Duke Forest</td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>9.51</td>
<td>0.00712</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>10.3</td>
<td>0.00639</td>
</tr>
<tr>
<td></td>
<td>Harvard Forest</td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>0.228</td>
<td>0.666</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>0.159</td>
<td>0.717</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>Duke Forest</td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>10.7</td>
<td>0.00481</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>12.2</td>
<td>0.00357</td>
</tr>
<tr>
<td></td>
<td>Harvard Forest</td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>0.398</td>
<td>0.573</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>0.570</td>
<td>0.505</td>
</tr>
</tbody>
</table>
MaxEnt calibrating variable abbreviations: $T_{an} = \text{mean annual temperature}; T_{qt} = \text{mean temperature during the warmest annual quarter}; T_{\text{max}} = \text{maximum annual temperature}$. Projected future distributions were developed using the CCCMA-CGCM2 climate model.

(Numerator degrees of freedom, denominator degrees of freedom) for predictors: Duke Forest maximal accumulation temperature = (1, 16); Duke Forest thermal accumulation slope = (1, 14); Harvard Forest maximal accumulation temperature and thermal accumulation slope = (1, 3).
APPENDIX F. Sample calculations of thermal accumulation slope.

Fig. F1. Sample calculations of thermal accumulation slope. The top left panel presents the thermal accumulation slope (± 1 SE) as a function of $CT_{\text{max}}$ for the 19 species at Duke Forest; the solid grey line indicates the slope of this regression, and the dashed grey lines indicate the
standard errors of the predicted values. The remaining panels present examples of the calculation of the thermal accumulation slope (the natural log of worker density as a function of the °C above ambient among the different warming chambers). Three species with different functional responses to warming are presented: a heat tolerant species (*Crematogaster lineolata*; red lines), a heat intolerant species (*Prenolepis impairs*; blue lines), and a heat insensitive species (*Aphaenogaster lamellidens*; green lines). The solid line is the thermal accumulation slope for each of these species, and the dashed lines indicate the standard errors of predicted values; these lines correspond with the point estimates (slope ± 1 SE) presented in the top left panel.
APPENDIX G. Phylogenetic model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions.

TABLE G1. Phylogenetic model summaries of ant responses to climate warming based on thermal tolerance and MaxEnt predictions.

<table>
<thead>
<tr>
<th>Site</th>
<th>Response</th>
<th>Predictor $^*$</th>
<th>$F$</th>
<th>$P$</th>
<th>$\lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duke Forest</strong></td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>9.29</td>
<td>0.00869</td>
<td>0.348</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MaxEnt $^{(T_{an})}$</td>
<td>0.893</td>
<td>0.361</td>
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</tr>
<tr>
<td></td>
<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>11.2</td>
<td>0.00485</td>
<td>0.136</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MaxEnt $^{(T_{an})}$</td>
<td>0.297</td>
<td>0.594</td>
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</tr>
<tr>
<td></td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>9.28</td>
<td>0.00871</td>
<td>0.359</td>
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<tr>
<td></td>
<td></td>
<td>MaxEnt $^{(T_{qt})}$</td>
<td>0.878</td>
<td>0.365</td>
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</tr>
<tr>
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<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>11.2</td>
<td>0.00482</td>
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<td></td>
<td></td>
<td>MaxEnt $^{(T_{qt})}$</td>
<td>0.767</td>
<td>0.396</td>
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<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>10.6</td>
<td>0.00583</td>
<td>0.358</td>
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<td></td>
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<td>MaxEnt $^{(T_{max})}$</td>
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<td>0.110</td>
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<tr>
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<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>12.2</td>
<td>0.00362</td>
<td>0.274</td>
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<tr>
<td></td>
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<td>MaxEnt $^{(T_{max})}$</td>
<td>2.04</td>
<td>0.175</td>
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<tr>
<td><strong>Harvard Forest</strong></td>
<td>maximal accumulation temperature</td>
<td>$CT_{max}$</td>
<td>0.00718</td>
<td>0.940</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td></td>
<td>MaxEnt $^{(T_{an})}$</td>
<td>0.0002</td>
<td>0.990</td>
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<tr>
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<td>thermal accumulation slope</td>
<td>$CT_{max}$</td>
<td>8.47</td>
<td>0.101</td>
<td>&lt;0.0001</td>
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<td>MaxEnt $^{(T_{an})}$</td>
<td>0.348</td>
<td>0.615</td>
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<td>maximal accumulation temperature</td>
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<td>MaxEnt $^{(T_{qt})}$</td>
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<td>$CT_{\text{max}}$</td>
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<td></td>
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<tr>
<td>maximal accumulation temperature</td>
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<td>0.305</td>
<td>0.636</td>
<td>&lt;0.0001</td>
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<td>MaxEnt ($T_{\text{max}}$)</td>
<td>4.07</td>
<td>0.181</td>
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<td>thermal accumulation slope</td>
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<td>5.73</td>
<td>0.139</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MaxEnt ($T_{\text{max}}$)</td>
<td>0.865</td>
<td>0.450</td>
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</tr>
</tbody>
</table>

*MaxEnt predictors: $T_{\text{an}}$ denotes mean annual temperature, $T_{\text{qt}}$ denotes mean temperature during the warmest quarter, and $T_{\text{max}}$ denotes maximum temperature during the warmest quarter.*

APPENDIX H. Ant worker density as a function of warming treatment at Harvard Forest.
FIG. H1. Ant worker density as a function of chamber delta (°C) at Harvard Forest. Symbols correspond with species identity: *Aphaenogaster rudis* (filled circles), *Camponotus pennsylvanicus* (open circles), *Formica subsericea* (filled squares), *Lasius alienus* (open squares), *Myrmica punctiventris* (filled triangles), *Temnothorax longispinosus* (open triangles).
APPENDIX I. Regressions of maximal accumulation temperature as functions of thermal tolerance and MaxEnt predictions.
Fig. 11. The predictive ability of thermal tolerance versus species distribution models in ant responses to warming at high and low latitudes: thermal accumulation slope (the slope, $\beta$, of the linear relationship between \(\ln(\text{cumulative worker density)}\) and $\Delta c$) as a function of (A,C) the critical thermal maximum ($CT_{\text{max}}$), and (B,D) MaxEnt prediction (the change in probability of occurrence across MaxEnt models based on current and future (2080) climate as defined by mean annual temperature) at (A,B) the low latitude site (Duke Forest), and (C,D) the high latitude site (Harvard Forest). Each point represents a single species; solid orange lines represent simple linear regressions (p-values indicate whether the slope is significantly different from zero), and dashed blue lines represent 95% confidence intervals.

**APPENDIX J.** Regressions of thermal accumulation slope (including standard errors) as functions of thermal tolerance and MaxEnt predictions.
Fig. J1. The predictive ability of thermal tolerance versus species distribution models in ant responses to warming at high and low latitudes: thermal accumulation slope (the slope, $\beta$, of the linear relationship between ln(cumulative worker density) and $\Delta c$) as a function of (A,C) the critical thermal maximum ($CT_{\text{max}}$), and (B,D) MaxEnt prediction (the change in probability of occurrence across MaxEnt models based on current and future (2080) climate as defined by mean annual temperature) at (A,B) the low latitude site (Duke Forest), and (C,D) the high latitude site (Harvard Forest). Each point represents a single species; solid orange lines represent simple linear regressions, and dashed blue lines represent 95% confidence intervals.