

Beyond thermal limits: comprehensive metrics of performance identify key axes of thermal adaptation in ants

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Summary

1. How species respond to temperature change depends in large part on their physiology. Physiological traits, such as critical thermal limits (CT_{max} and CT_{min}), provide estimates of thermal performance but may not capture the full impacts of temperature on fitness. Rather, thermal performance likely depends on a combination of traits—including thermal limits—that vary among species.

2. Here, we examine how thermal limits correlate with the main components that influence fitness in ants. First, we compare how temperature affected colony survival and growth in two ant species that differ in their responses to warming in the field—*Aphaenogaster rudis* (heat-intolerant) and *Temnothorax curvispinosus* (heat-tolerant). We then extended our study to compare CT_{max} , thermal requirements of brood and yearly activity season among a broader set of ant species.

3. While thermal limits were higher for workers of *T. curvispinosus* than *A. rudis*, *T. curvispinosus* colonies also required higher temperatures for survival and colony growth. This pattern generalized across 17 ant species, such that species whose foragers had a high CT_{max} also required higher temperatures for brood development. Finally, species whose foragers had a high CT_{max} had relatively short activity seasons compared with less heat-tolerant species.

4. The relationships between CT_{max} , thermal requirements of brood and seasonal activity suggest two main strategies for growth and development in changing thermal environments: one where ants forage at higher temperatures over a short activity season and another where ants forage at lower temperatures for an extended activity season. Where species fall on this spectrum may influence a broad range of life-history characteristics and aid in explaining the current distributions of ants as well as their responses to future climate change.

Key-words: climate change, critical thermal limits, development, phenology, social insects, thermal adaptation

Introduction

For millions of years, species have faced changes in climate that have shaped their evolution and their biology (Crowley & North 1988; Petit *et al.* 1999; Davis, Shaw & Etterston 2005). The result is that species display differences in key traits that can be used to estimate thermal performance and predict how species will respond to climate change in the future (Chown, Gaston & Robinson 2004;

Deutsch *et al.* 2008; Buckley & Kingsolver 2012; Sunday, Bates & Dulvy 2012; Diamond *et al.* 2013). Thermal performance is often estimated using simple metrics, such as critical thermal limits (CT_{max} and CT_{min}), which define the upper and lower temperatures at which a species can operate (Huey & Stevenson 1979). But even within these bounds, changes in temperature can influence a wide range of factors that impact fitness (Kingsolver 2009; Hofmann & Todgham 2010; Schulte, Healy & Fanguie 2011; Chevin, Collins & Lefèvre 2013). What is missing for most species is an understanding of how thermal limits correlate with

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other traits that impact fitness and how these combine to characterize general strategies for how organisms deal with climate variation.

Social insects provide a unique opportunity to compare thermal limits with other factors that influence fitness because they live in colonies with overlapping developmental stages that inhabit the same environment at the same time. This allows a direct comparison of the relationship between thermal traits of adults and those of earlier developmental stages. Ants, in particular, have served as models to study the impacts of temperature on animal populations (Jenkins *et al.* 2011; Diamond *et al.* 2012b; Warren & Chick 2013; Kaspari *et al.* 2015; Verble-Pearson, Yanoviak & Gifford 2014; Diamond *et al.* 2016) due to their ubiquity and the important roles they play in many ecosystems (Folgarait 1998). Yet, most studies on ant thermal performance have focused exclusively on thermal limits of mature foragers—worker ants that leave the nest to find food (Cerdá, Retana & Cros 1998; Diamond *et al.* 2012b; Stuble *et al.* 2013a; Kaspari *et al.* 2015). The focus on thermal traits of foragers neglects the social dimension of ant colony performance. The performance of an entire colony, which is the unit of selection in social insects, depends not only on how temperature impacts mature foragers but also on how temperature impacts survival and development of other colony members, including brood.

When resources are not limiting, colony growth depends on three main factors: egg-laying rate, worker mortality rate and brood development time (Asano & Cassill 2011, 2012). Increased temperatures tend to speed up egg-laying rates (Abril, Oliveras & Gómez 2008) and brood development (Porter 1988; Kipyatkov *et al.* 2004; Kipyatkov, Lopatina & Imamgaliev 2005; Abril, Oliveras & Gómez 2010; Karlick *et al.* 2016) while simultaneously increasing worker mortality (Calabi & Porter 1989). How these components combine to determine colony performance and fitness within a single ant species over a range of different temperatures is unknown. Further, it is unclear if and how the thermal requirements of immature ants (brood) relate to thermal limits of mature ants (foragers). Finally, we lack an understanding of how relationships between brood and adult thermal traits may differ between more thermophilic species and thermophobic species.

Here, we investigated how thermal limits of mature foragers correlate with the other main components that influence fitness for ant colonies. We focused first on two ant species—*Aphaenogaster rudis* and *Temnothorax curvispinosus*—that are among the most common ectotherms in eastern forests of North America (Pearse 1946; King, Warren & Bradford 2013; Stuble *et al.* 2013b). Foragers of *A. rudis* have a relatively low CT_{max} and are active at cooler temperatures, while foragers of *T. curvispinosus* have a relatively high CT_{max} and are active at warmer temperatures (Diamond *et al.* 2012a; Pelini *et al.* 2012; Stuble *et al.* 2013a). Based on differences in CT_{max} , we predicted that colony survival and growth would be higher for *A. rudis* at cooler temperatures than for *T. curvispinosus*. We then

extended our study to include additional ant species that co-occur with *A. rudis* and *T. curvispinosus* across much of the eastern United States. For these species, we compared the CT_{max} of foragers with the thermal dependence of pupal development time. Again, we predicted that species whose foragers had a higher CT_{max} would require higher temperatures for brood development. Finally, we compared the relationship between CT_{max} and seasonal activity patterns for a subset of species for which we had previously tracked yearly activity patterns as part of a long-term, field-based warming experiment (Pelini *et al.* 2011). We predicted that species with a higher CT_{max} would have a shorter activity season based on limits to colony growth during cold times of the year.

Materials and methods

STUDY SPECIES AND COLONY MAINTENANCE

We collected colonies of *A. rudis* (a species complex, Umphrey (1996)) and *T. curvispinosus* between April and June 2013. Colonies of *A. rudis* were collected from 13 populations across their range (Tables S1 and S2, Supporting Information), while *T. curvispinosus* colonies were collected from a single population in Raleigh, NC, USA (35-7639, -78-6779, 95 m). We additionally collected colonies of 12 ant species that co-occur with *A. rudis* and *T. curvispinosus* to compare broader patterns of thermal trait variation (Tables 1 and S3). Colonies were housed in artificial nest boxes with a plaster floor and a glass covered nest chamber. The plaster in each nest was moistened daily with distilled water to maintain humidity, and we fed colonies an artificial diet designed specifically for ants (Bhatkar & Whitcomb 1970) that we changed three times per week and supplemented with freeze-killed beetle larvae (*Zophobas morio*) and vials containing 20% sucrose solution. Colonies of all species were held under common laboratory conditions (standard long-days photoperiod; ~25 °C) for at least 2 weeks prior to experimental treatments.

EXPERIMENTAL REARING TEMPERATURES

We measured thermal traits at four mean temperatures: 20, 23, 26 and 29 °C (Fig. S1). These temperatures were chosen to cover the likely range of nest temperatures colonies could tolerate before experiencing complete mortality or entering diapause (Porter 1988; Southerland 1988; Abril, Oliveras & Gómez 2010; Kipyatkov & Lopatina 2015). Colonies were maintained on a 14 h : 10 h light-dark cycle in temperature-controlled growth chambers (walk-in chambers measuring 2.4 m width × 1.2 m depth × 2.1 m height), and temperature in each chamber was programmed to fluctuate diurnally around the mean temperature treatments. Chambers were initially programmed with a high daily temperature fluctuation (mean ± 3 °C) with a 1 °C change every 2 h (Fig. S1a). Due to high colony mortality during the first 2 weeks (e.g., ~40% colony mortality for *A. rudis* at 29 °C), the temperature fluctuations were reduced to ±1.5 °C with a 0.5 °C change every 2 h (Fig. S1b).

TRAIT MEASUREMENTS: COLONY SURVIVAL AND GROWTH OF *A. RUDIS* AND *T. CURVISPINOSUS*

We quantified colony survival and a range of colony growth traits at each rearing temperature for a total of 54 *A. rudis* colonies and

Table 1. Ant thermal traits and activity season

Species	CT _{max} (°C) SD, <i>n</i>	Pupal development time (days)				Activity season Length (days) IQR (Julian date range)
		20°C	23°C	26°C	29°C	
<i>Crematogaster lineolata</i>	47.7 ± 0.94, 10	42	21	14	10	51.75 179.25–231
<i>Temnothorax curvispinosus</i>	46.3 ± 1.26, 4	32	21	13	12	77.25 179.25–256.5
<i>Formica subsericea</i>	46.2 ± 0.45, 5	21	16	11	10	74 127–201
<i>Monomorium minimum</i>	46.0 ± 1.07, 8	40	20	11	10	–
<i>Temnothorax longispinosus</i>	45.2 ± 1.14, 10	32	20	14	12	–
<i>Tetramorium sp. E</i>	44.9 ± 0.57, 10	35	19	12	11	–
<i>Tapinoma sessile</i>	44.4 ± 2.40, 9	27	17	10	9	–
<i>Solenopsis invicta</i>	43.7 ± 0.78, 9	39	25	12	9	–
<i>Lasius alienus</i>	42.2 ± 1.14, 10	25	17	12	11	–
<i>Aphaenogaster rudis</i>	41.3 ± 0.95, 10	18	15	11	9	101 167–268
<i>Aphaenogaster lamellidens</i>	40.8 ± 0.63, 10	27	19	11	11	95.5 127.5–223
<i>Linepithema humile</i>	40.4 ± 1.94, 9	29	14	9	9	–
<i>Solenopsis molesta</i>	40.3 ± 1.03, 6	23	20	17	11	100 204.25–304.25
<i>Brachyponera chinensis</i>	38.1 ± 1.20, 10	30	19	12	12	–

25 *T. curvispinosus* colonies (data for all traits were not measured in every colony, and sample sizes are reported independently where they diverge). Colonies were standardized to one queen with 100 workers for *A. rudis* and one queen with 50 workers for *T. curvispinosus*. These colony sizes were chosen because most healthy colonies collected in the field had at least 100 workers for *A. rudis* and 50 workers for *T. curvispinosus*. Nine *A. rudis* colonies had fewer than 100 workers when originally collected, so we included all available workers for these colonies (mean ± SD of colony sizes were 95.6 ± 11.9). All eggs, larvae and pupae were removed before the start of the experiment, so egg-laying rate and duration of each brood stage could be quantified.

Colonies were checked daily over 12 weeks to assess colony survival and quantify colony growth traits (egg-laying rate, worker mortality rate, brood development time and brood mortality). Colony survival was determined based on whether a colony had a living queen, and a colony was considered dead on the date the queen died even if some workers remained. Egg-laying rate was determined by counting the number of eggs in a colony on a single day (within 4–18 days after the start of the experiment) and then dividing this by the number of days elapsed since the first eggs were observed. Worker mortality rate was determined by counting the number of workers present in a colony on a single day (within 4–18 days after the start of the experiment), subtracting this from the initial colony size, and then dividing this by the number of days elapsed since the start of the experiment. The duration of each brood stage (egg, larval and pupal) was determined based on the number of days between the first appearance of a particular brood stage and the appearance of the subsequent stage. Finally, brood mortality was determined based on the number of colonies that produced a particular brood stage but failed to rear brood to the subsequent stage.

TRAIT MEASUREMENTS: CT_{MAX}, PUPAL DURATION AND ACTIVITY SEASON

We measured CT_{max}, pupal duration and activity season for a broader set of ant species that also included *A. rudis* and *T. curvispinosus* (Table 1; note, only NC populations of *A. rudis* were used

for these comparisons). CT_{max} was estimated on adult individuals taken from the field that were acclimated in the laboratory at 25 °C for at least 4 weeks prior to CT_{max} assessment. Individuals were placed into 2-mL culture tubes that were inserted into a heat block set to 36 °C, and temperature was increased stepwise at a rate of 1 °C every 5 minutes. We defined CT_{max} as the temperature at which individuals lost muscle coordination and could no longer right themselves (Lutterschmidt & Hutchison 1997). We tested one individual per colony and 4–10 colonies for each species.

We measured pupal duration for 14 species at each rearing temperature (20, 23, 26, 29 °C; Table S3). Pupal duration was determined based on the number of days between the first appearance of pupae in a colony and the date when new workers enclosed. We included data for three additional ant species (*Lasius niger*, *Myrmica rubra* and *Myrmica ruginodis*) for which pupal development time at 20 °C was previously published (Kipyatkov, Lopatina & Imamgaliev 2005; Kipyatkov & Lopatina 2015). CT_{max} for each of these species was not known, so we used the average CT_{max} for each genus [*Lasius*: average = 38.7 °C, SE = 0.04; *Myrmica*: average = 39.4 °C, SE = 0.03 (Diamond *et al.* 2012b)].

We measured activity season of six species in a community we have monitored at Duke Forest (36-0355, –79-0775, 180 m) as part of a long-term climate warming study (Pelini *et al.* 2011). These six species, which included *A. rudis* and *T. curvispinosus*, were the most abundant at Duke Forest and the only species collected in high enough numbers for determining activity season. Ants were collected from monthly pitfall traps over 5 years from 2010 to 2014, and all individuals were identified to species (we focused on ants collected only from unheated control plots). The length of the activity season was estimated as the interquartile range of annual occurrences in monthly pitfalls pooled over the 5-year monitoring interval as proxies for annual first appearance and last appearance.

STATISTICAL ANALYSES

We initially tested for local adaptation in thermal traits for *A. rudis* by adding mean annual temperature (MAT) of source

populations as a covariate in our models [MAT was determined using 30-arc second grid cell maps from WorldClim (Hijmans *et al.* 2005)]. However, MAT was never statistically significant, so we report models without this covariate for simplicity.

For comparing thermal responses of *A. rudis* and *T. curvispinosus*, we treated rearing temperature as a categorical variable because thermal responses are not always linear (Kingsolver 2009). For colony survival, we used a generalized linear model (GLM) with a binomial distribution and a logit link function; whether or not a colony survived at the end of 12 weeks was the dependent variable, and species, rearing temperature and their interaction were the independent variables. For egg-laying rate and worker mortality, we used ANOVA with egg-laying rate and worker mortality as the dependent variables (respectively) and species, rearing temperature and their interaction as the independent variables. For pairwise comparisons, we used Tukey's HSD. Data for egg-laying rate and worker mortality were ln-transformed to meet normality assumptions.

For comparisons of brood development time between *A. rudis* and *T. curvispinosus*, we used hyperbolic functions fit to development time (in days) across rearing temperatures. These models are typical for studies of development in insects and other ectotherms and provide a common framework for comparing performance curves among species (Wagner *et al.* 1984; Ratte 1985; Kipyatkov & Lopatina 2015). For comparisons of stage-specific brood mortality, we used a generalized linear model (GLM) with a binomial distribution and a logit link function; brood mortality at each stage (egg, larval and pupal) was the dependent variable, and species, rearing temperature and their interaction were the independent variables.

Finally, we used a generalized least squares modelling framework to compare the relationship between CT_{max} and pupal development time as well as CT_{max} and activity season length. We accounted for the non-independence arising from the shared evolutionary history of species by scaling the model covariance by the degree of phylogenetic signal, i.e., the maximum likelihood branch transformation (Pagel's λ ; Pagel 1999) given the data and the model (Orme *et al.* 2013). We used the phylogeny of Moreau & Bell (2013) (Fig. S2), but because this phylogeny is resolved only to the level of genus, we treated unknown species relationships as terminal polytomies (Lessard *et al.* 2009; Liu *et al.* 2016). For pupal development time, pupal durations at each rearing temperature (20, 23, 26 and 29°C) were the response variables, and CT_{max} was the predictor variable. For comparing CT_{max} with activity season, the number of days active per year (Table 1) was the response variable, and CT_{max} was the predictor variable. Phylogenetic generalized least squares comparisons were performed using R version 3.1.1, and all other analyses were performed in JMP Pro 12.0.1 (SAS Institute Inc., Cary, NC, USA).

Results

IMPACT OF TEMPERATURE ON COLONY SURVIVAL

The impact of temperature on colony survival differed between *A. rudis* and *T. curvispinosus* [GLM (binomial, logit link function), $N_{A. rudis}$ = 54 colonies, $N_{T. curvispinosus}$ = 25 colonies, d.f. = 3; χ^2 = 18.12, P = 0.0004]. *Aphaenogaster rudis* had higher colony mortality at warm temperatures, while *T. curvispinosus* had higher colony mortality at cool temperatures (Fig. 1).

IMPACT OF TEMPERATURE ON COLONY GROWTH

The impact of temperature on both egg-laying rate and worker mortality rate differed between *A. rudis* and

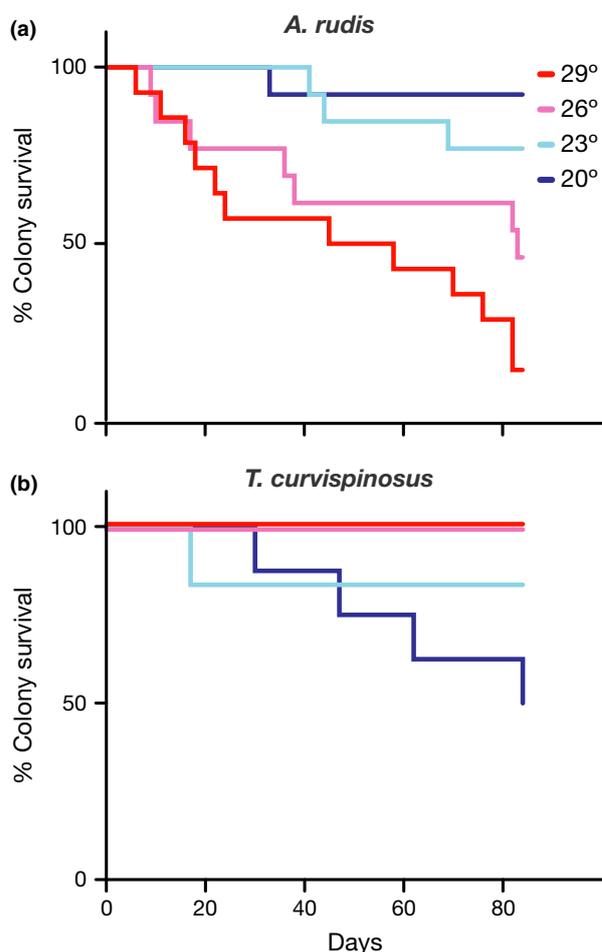


Fig. 1. Colony survival as a function of temperature for *Aphaenogaster rudis* and *Temnothorax curvispinosus*. Each species differed in their response to temperature by the end of the experiment (P = 0.0004), with *A. rudis* exhibiting higher mortality at warm temperatures and *T. curvispinosus* exhibiting higher mortality at cool temperatures. [Colour figure can be viewed at wileyonlinelibrary.com]

T. curvispinosus, which was indicated by a significant Species \times Rearing temperature interaction for egg-laying rate (ANOVA, $N_{A. rudis}$ = 43 colonies, $N_{T. curvispinosus}$ = 23 colonies, d.f. = 3; F = 3.01, P = 0.037) and worker mortality rate (ANOVA, natural log transformed, $N_{A. rudis}$ = 28 colonies, $N_{T. curvispinosus}$ = 20 colonies, d.f. = 3; F = 5.82, P = 0.0021). Egg-laying rate was relatively insensitive to temperature for *A. rudis*, but *T. curvispinosus* had a higher egg-laying rate at the relatively warm rearing temperature of 26 °C (Fig. 2a). However, there were no significant differences between any rearing temperature–species comparison (Tukey HSD pairwise comparisons, P > 0.05). With respect to worker mortality, the strongest impact was on *A. rudis*, where there was a nearly three-fold increase in worker mortality at 29 °C for *A. rudis* compared with cooler rearing temperatures (Fig. 2b). Worker mortality was significantly higher for *A. rudis* at 29 °C than worker mortality for *T. curvispinosus* at 20 °C or 23 °C (Tukey HSD pairwise comparisons; 20 vs. 29 °C, P = 0.0001; 23 vs. 29 °C, P = 0.04). In contrast, *T. curvispinosus* had

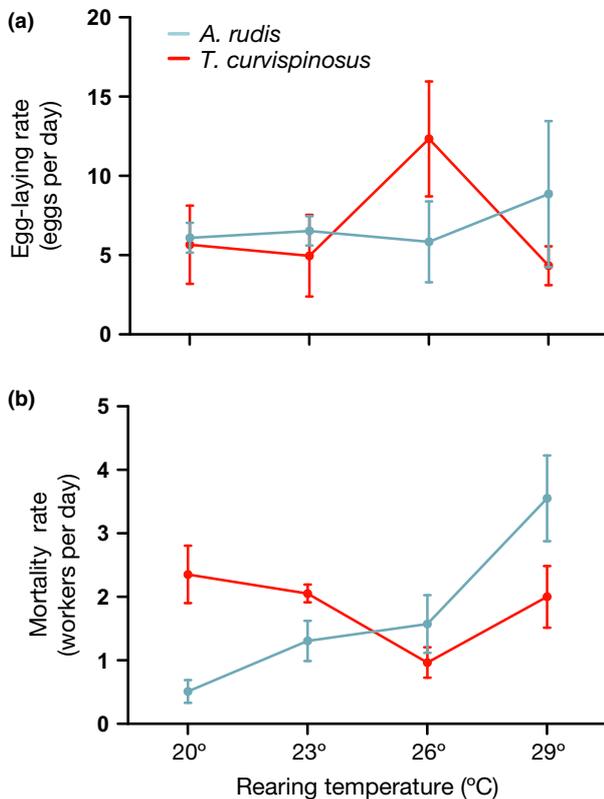


Fig. 2. Egg-laying rate and worker mortality rate (mean \pm SE) across rearing temperatures for *Aphaenogaster rudis* and *Temnothorax curvispinosus*. (a) Egg-laying rate differed between species across rearing temperatures ($P = 0.037$), with *T. curvispinosus* exhibiting a peak in egg-laying rate at 26 °C. (b) Worker mortality rate also differed between species across rearing temperatures ($P = 0.0021$), with *A. rudis* exhibiting an increase in worker mortality at the warmest treatment (29 °C). [Colour figure can be viewed at wileyonlinelibrary.com]

significantly higher mortality than *A. rudis* at 20 °C (Tukey HSD pairwise comparisons, $P = 0.018$).

With respect to brood development, *T. curvispinosus* had shorter (i.e., faster) egg and larval development times across most temperatures, but *A. rudis* was able to maintain larval development at cooler temperatures than *T. curvispinosus* (Fig. 3a–c). Larval development halted below 23 °C for *T. curvispinosus*, while *A. rudis* maintained larval development down to at least 20 °C. Each species also differed in how temperature impacted brood mortality, which was evidenced by a significant interaction between species and rearing temperature for egg mortality [GLM (binomial, logit link function), $N_{A. rudis} = 52$ colonies, $N_{T. curvispinosus} = 25$ colonies, d.f. = 1; $\chi^2 = 7.65$, $P = 0.0057$] and larval mortality [GLM (binomial, logit link function), $N_{A. rudis} = 30$ colonies, $N_{T. curvispinosus} = 24$ colonies, d.f. = 1; $\chi^2 = 6.68$, $P = 0.0097$]. Egg mortality was low for both *A. rudis* and *T. curvispinosus* at cool temperatures, but only 25% of *A. rudis* colonies were able to successfully rear eggs at the two warmest temperatures (Fig. 3d). Likewise, larval mortality increased with temperature for *A. rudis*, while *T. curvispinosus* had increased larval mortality at

the two coolest temperatures (Fig. 3e). There was no significant interaction between species and rearing temperature for pupal mortality [GLM (binomial, logit link function), $N_{A. rudis} = 42$ colonies, $N_{T. curvispinosus} = 19$ colonies, d.f. = 1; $\chi^2 < 0.0001$, $P = 1.0$], and pupal mortality was generally low for both species across all rearing temperatures (Fig. 3f).

RELATIONSHIP BETWEEN CT_{MAX} AND THERMAL REQUIREMENTS OF BROOD

At warm temperatures, pupal development times of all species converged on roughly the same maximum value (mean: 10.5 days, range: 8.6–11.8 days; Fig. 4a). As temperatures decreased, species with a higher CT_{max} required a longer time to complete development (Fig. 4b), which was evidenced by a significant positive relationship between CT_{max} and pupal duration at 20 °C [GLM (normal, identity link function), $n = 14$ species, d.f. = 1; $\chi^2 = 4.15$, $P = 0.0416$]. The strength of this relationship increased when data were added for three additional species for which development times at 20 °C were reported in the literature [GLM (normal, identity link function), $n = 17$ species, d.f. = 1; $\chi^2 = 6.88$, $P = 0.0087$]. At warmer temperatures, there were no significant relationships between CT_{max} and pupal development times among the 14 species in our study [GLM (normal, identity link function), $n = 14$ species, d.f. = 1: 29 °C, $\chi^2 = 0.18$, $P = 0.67$; 26 °C, $\chi^2 = 0.04$, $P = 0.84$; 23 °C, $\chi^2 = 1.18$, $P = 0.28$]. Because phylogenetic signal was estimated to be very low in our PGLS analyses, our results from the phylogenetically corrected models were qualitatively similar to our uncorrected models, so we present only the GLMs for simplicity. Our PGLS models estimated phylogenetic signal, λ , as 0 in each model of development time, and no estimates of phylogenetic signal were significantly different from 0. One caveat here is that we do not have the recommended number of species (>20) to estimate phylogenetic signal (Blomberg *et al.* 2003), so while our results suggest a weak phylogenetic signal in ant development time, this may not be the case were more species to be compared.

RELATIONSHIP BETWEEN CT_{MAX} AND ACTIVITY SEASON

Based on the relationship between CT_{max} and thermal requirements for brood development, we predicted that species with a high CT_{max} would also have a shorter activity season as cold temperatures would limit the period over which brood could grow (Fig. 5a). As a test of this prediction, we compared activity season for the six most common species at our long-term study site at Duke Forest, which included *A. rudis* and *T. curvispinosus*. There was a significant negative relationship between CT_{max} and activity season for these six species [GLM (normal, identity link function), $n = 6$ species, d.f. = 1; $\chi^2 = 12.90$, $P = 0.0003$], which supported our prediction (Fig. 5b). For the two focal species from the common garden

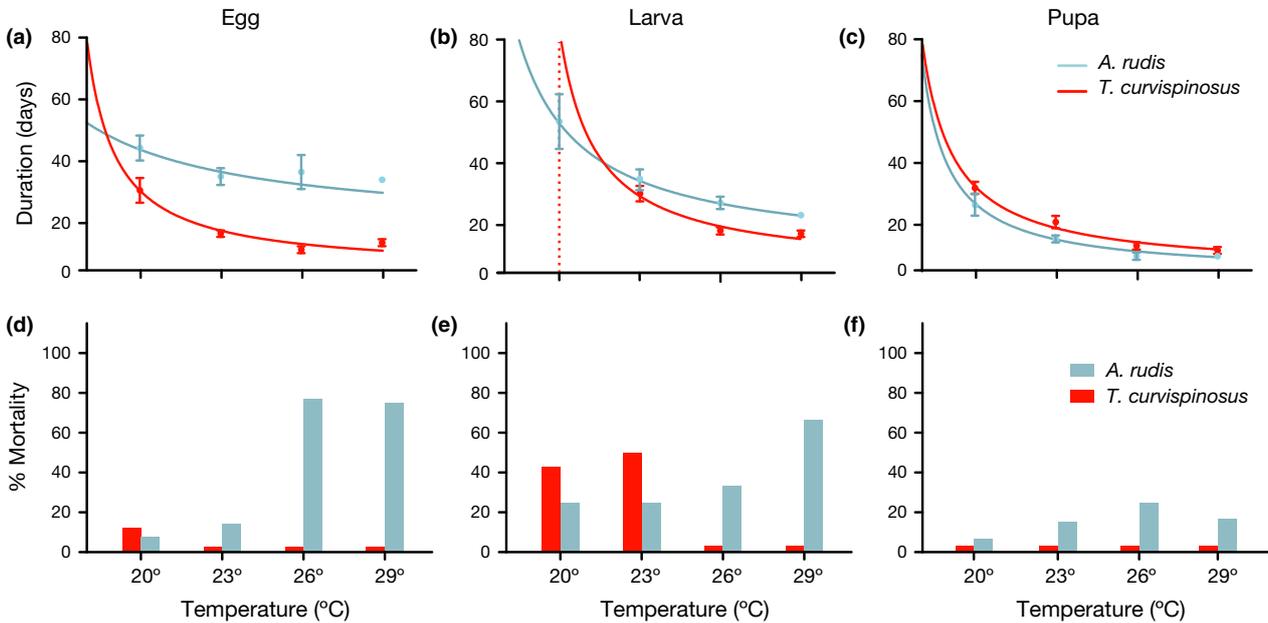


Fig. 3. Stage-specific development times and brood mortality as a function of temperature for *Aphaenogaster rudis* and *Temnothorax curvispinosus*. (a–c) Development time for egg, larval and pupal stages across rearing temperatures (Mean \pm SE) fitted with hyperbolic functions. Larvae of *T. curvispinosus* went into diapause at 20° (dotted line). (d–f) The percent of colonies that failed to rear brood at each stage successfully. Each species differed in their response to temperature with respect to egg mortality ($P = 0.0057$) and larval mortality ($P = 0.0097$), while there was little pupal mortality for either species across temperatures. [Colour figure can be viewed at wileyonlinelibrary.com]

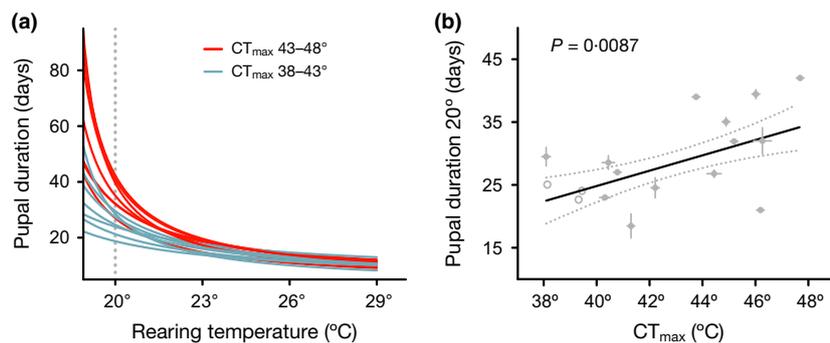


Fig. 4. Relationship between pupal development time and CT_{max} among species. (a) Pupal development time across rearing temperatures for 14 co-occurring ant species fitted with hyperbolic functions. (b) Relationship between CT_{max} and pupal duration at 20° (points show mean \pm SE) fitted with a linear function plus 95% confidence intervals ($y = 1.23x - 24$; $R^2 = 0.27$). Open circles indicate three species for which pupal development time and CT_{max} were taken from the literature. [Colour figure can be viewed at wileyonlinelibrary.com]

experiment, activity season was roughly 30% longer for heat-intolerant *A. rudis* compared with heat-tolerant *T. curvispinosus*. Like our phylogenetic models of development time, we found no evidence for phylogenetic signal in our models of activity season ($\lambda = 0$), so we present only the GLM for simplicity.

Discussion

Thermal limits are often used to predict how species will respond to climate warming, but temperature affects a wide range of traits that can impact fitness (Hofmann & Todgham 2010). We found a correlation between the thermal limits of ants and how temperature affected the

other main components that influence colony survival and growth. In the species we studied, thermal performance was driven by a relationship between thermal limits of adult workers and thermal requirements of brood, such that species whose foragers had higher thermal limits—and could forage under hotter conditions—also required higher temperatures for brood development. Conversely, species whose foragers could not tolerate high temperatures were able to maintain relatively fast development rates at cool temperatures and remain active over a longer growing season. Taken together, these results highlight that thermal performance depends on an interaction between multiple, thermally dependent traits that ultimately affect fitness.

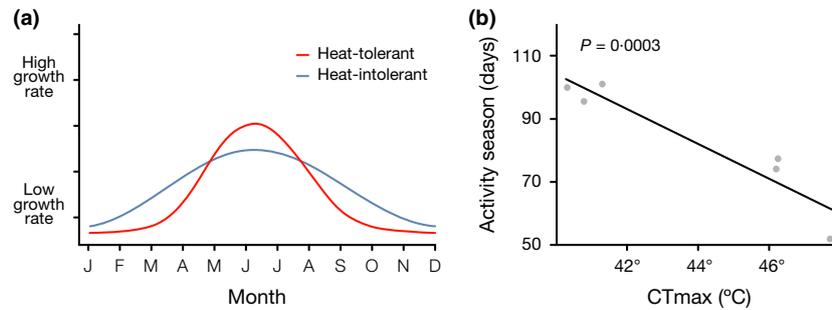


Fig. 5. Negative relationship between CT_{max} and activity season. (a) Conceptual figure illustrating predicted colony growth rates for heat-tolerant and heat-intolerant species. Heat-tolerant species are predicted to grow fast over a short season, while heat-intolerant species are predicted to extend growth over a longer portion of the year. Letters on x-axis indicate months, and y-axis represents relative differences in colony growth rate. (b) Activity season plotted as a function of CT_{max} for six species present at Duke Forest showing a negative relationship between CT_{max} and number of days active per year. [Colour figure can be viewed at wileyonlinelibrary.com]

COLONY SURVIVAL

We predicted that optimal temperatures for colony survival would be cooler for *Aphaenogaster rudis* (heat-intolerant) than for *T. curvispinosus* (heat-tolerant) based on differences in their thermal limits, and our results supported this prediction: colony survival was highest at relatively cool temperatures for *A. rudis* (20–23 °C), while survival was highest at relatively warm temperatures for *T. curvispinosus* (26–29 °C). Compared with CT_{max} and CT_{min} of individual workers, however, the range of temperatures over which colonies could survive was relatively narrow. The CT_{max} of *A. rudis* workers is 41 °C, but colonies of *A. rudis* experienced 90% mortality at only 29 °C. Likewise, colonies of *T. curvispinosus* experienced nearly 40% mortality at 20 °C, which is only slightly cooler than standard room temperature and consistent with relatively high overwintering mortality of *Temnothorax* colonies in the field (Mitrus 2013).

The sensitivity of ant colonies to minor deviations in temperature suggests that temperatures need not exceed thermal limits to negatively impact fitness. While CT_{max} is a useful metric for predicting how species will respond to temperature increases, survival is influenced both by the intensity and the duration of thermal stress (Rezende, Castañeda & Santos 2014). Our results and those of others (Magozzi & Calosi 2015; McDonnell & Chapman 2015) suggest that moderate increases in temperature can negatively impact performance, especially when individuals are exposed to chronic increases in temperature. Caution is therefore warranted when using CT_{max} alone to predict the vulnerability of species to climate warming. Nevertheless, for *A. rudis* and *T. curvispinosus*, CT_{max} is correlated with responses to chronic thermal stress and can provide an estimate of relative thermal performance.

COLONY GROWTH

Similar to results for colony survival, *A. rudis* performed better at cool temperatures in terms of growth, while *T. curvispinosus* performed better at warmer temperatures.

Egg-laying rates were relatively insensitive to temperature for both species, but *T. curvispinosus* had slightly higher egg-laying rates at the moderately warm temperature of 26 °C. The impact of temperature on worker mortality was more pronounced, particularly for *A. rudis*, where mortality increased from less than one worker per day at 20 °C to over three workers per day at 29 °C. The major difference in how temperature affected these species was with respect to brood. *Temnothorax curvispinosus* brood developed faster at warm temperatures, while *A. rudis* developed faster at cool temperatures where *T. curvispinosus* brood went into diapause. A comparison of CT_{max} and pupal duration across a broader set of ant species revealed a similar trend, with heat-tolerant species growing slower at low temperatures. The difference in growth at low temperatures is noteworthy because, all things being equal, heat-tolerant species should grow faster during the warmest months of the year, while heat-intolerant species should grow faster during cooler months.

The positive relationship between CT_{max} and pupal duration at 20 °C suggests that CT_{max} —in addition to providing information about lethal temperatures—can also provide information about the performance of species at sublethal temperatures. If greater heat tolerance is consistently associated with reduced fitness at moderate and lower temperatures, the utility of CT_{max} for predicting responses to climate change could be much greater. Moreover, the correlation between CT_{max} and thermal traits of brood suggests that thermal limits of adults may provide information about thermal responses of early developmental stages. This at least may be true for social insects, where adult and brood stages live in the same nest and experience relatively similar thermal conditions. Solitary insects often have complex life cycles where distinct life stages experience different environmental conditions, and in these cases, each development stage may have different thermal requirements and exhibit distinct thermal tolerances (Kingsolver *et al.* 2011).

One aspect of thermal performance we did not test was the impact of acclimation on CT_{max} . How species respond to climate change may depend on their ability to acclimate

(Calosi, Bilton & Spicer 2008; Somero 2010), but it is not clear if CT_{max} is highly plastic in ants within species. A study on Argentine ants found little effect of acclimation on CT_{max} (Jumbam *et al.* 2008), and we found low variation in CT_{max} among *A. rudis* populations ranging from South Carolina to Maine, USA (mean CT_{max} = 41.6 °C, range = 40.5–43 °C), and no relationship with MAT of source populations (C.A. Penick, S.E. Diamond, N.J. Sanders & R.R. Dunn unpubl. results; but see Cahan *et al.* 2017). In fact, there appears to be relatively low acclimation in thermal tolerance in general among ectotherms (Gunderson & Stillman 2015).

THERMAL STRATEGIES IN ANTS AND ECOLOGICAL IMPLICATIONS

A complete model of the thermal performance for ants should include information about the thermal limits of foragers as well as the impacts of temperature on brood. Our results suggest that these two aspects of thermal performance are correlated, in that species whose workers can tolerate higher temperatures also require higher temperatures for brood development. On one hand, alignment between brood and worker thermal responses could provide an advantage in that peak rates of brood development will occur at the same time foragers are most active. But when considering seasonal variation in temperature, heat-tolerant species will have limited growth in cooler months. In contrast, the ability of *A. rudis* and other heat-intolerant species to maintain brood development at cool temperatures should allow them to increase their growing season in regions where the number of warm days is limited. We found support for this based on a comparison of CT_{max} and activity season among six species from a single site in North Carolina: species whose foragers had a higher CT_{max} were active for a shorter period of the year. A previous study also found that *A. rudis* had a relatively long activity season compared with two other co-occurring species (Bewick *et al.* 2014).

The relationship between CT_{max} and activity season suggests two strategies ants adopt when dealing with their thermal environment: species either forage in the heat and grow fast over a short season, or avoid the heat and extend growth over a longer portion of the year (Fig. 5). While each of these strategies represents the opposite end of a continuum, they provide a framework for comparing thermal responses among species. For heat-tolerant species, colony growth is likely limited by the availability of heat to stimulate brood development, so heat-tolerant species may be more likely to nest in open areas exposed to sunlight, invest heavily in brood thermoregulation and/or build nests that are better at capturing solar heat. Alternatively, heat-intolerant species are more likely to be limited by their ability to gather resources when outside temperatures are high. Therefore, competition may play a larger role in limiting the performance of heat-intolerant species, especially when competing with heat-tolerant species for food.

The different strategies species use to deal with their thermal environment have implications for how climate warming will affect ant communities, especially in temperate regions with strong seasonal temperature variation. While heat-intolerant species may be able to escape high temperatures by moving deeper underground (Jones & Oldroyd 2006; Penick & Tschinkel 2008), they may face increased competition for resources that will ultimately reduce their performance under thermally stressful environments. These biotic interactions not only mediate coexistence in warm conditions (e.g., Cerdá, Retana & Cros 1997; Wittman *et al.* 2010) but they are likely to play a major role in setting current range limits for ants and determining their response to ongoing climate change.

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Data accessibility

All data included in the manuscript have been deposited at Dryad Digital Repository <https://doi.org/10.5061/dryad.sd64q> (Penick *et al.* 2016).

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Supporting Information

Details of electronic Supporting Information are provided below.

Table S1. Collection information for populations of *Aphaenogaster rudis* (USA).

Table S2. Sample size (number of colonies) across temperature treatments (°C).

Table S3. Development time functions.

Fig. S1. Experimental temperature treatments.

Fig. S2. Ant phylogenies used for PGLS analyses comparing (a) CT_{max} with pupal development time at 20 °C and (b) CT_{max} with activity season (phylogenies reconstructed from Moreau & Bell (2013)).