



## Research Paper

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### Abstract

Global warming is exposing many organisms to severe thermal conditions and is having impacts at multiple levels of biological organisation, from individuals to species and beyond. Biotic and abiotic factors can influence organismal thermal tolerance, shaping responses to climate change. In eusocial ants, thermal tolerance can be measured at the colony level (among workers within colonies), the population level (among colonies within species), and the community level (among species). We analysed critical thermal maxima ( $CT_{max}$ ) across these three levels for ants in a semiarid region of northeastern Brazil. We examined the individual and combined effects of phylogeny, body size (BS), and nesting microhabitat on community-level  $CT_{max}$  and the individual effects of BS on population- and colony-level  $CT_{max}$ . We sampled 1864 workers from 99 ant colonies across 47 species, for which we characterised  $CT_{max}$ , nesting microhabitat, BS, and phylogenetic history. Among species,  $CT_{max}$  ranged from 39.3 to 49.7°C, and community-level differences were best explained by phylogeny and BS. For more than half of the species,  $CT_{max}$  differed significantly among colonies in a way that was not explained by BS. Notably, there was almost as much variability in  $CT_{max}$  within colonies as within the entire community. Monomorphic and polymorphic species exhibited similar levels of  $CT_{max}$  variability within colonies, a pattern not always explained by BS. This vital intra- and inter-colony variability in thermal tolerance is likely allows tropical ant species to better cope with climate change. Our results underscore why ecological research must examine multiple levels of biological organisation.

### Introduction

Temperature governs many biological processes, affecting different levels of biological organisation (Verberk *et al.*, 2016). Changes in temperature can have profound effects on the functioning of cells, tissues, and organ systems (Brown *et al.*, 2004). At the organismal level, changes in environmental temperature can directly affect reproduction, growth, and survival (Deutsch *et al.*, 2008). Consequently, temperature can determine the abundance and dynamics of species populations within communities and, ultimately, species distribution ranges (Bujan *et al.*, 2020). Given that global temperature has increased considerably in recent decades as a result of climate change (IPCC, 2021), it is paramount to understand the possible impacts of temperature increases at different levels of biological organisation (Parr and Bishop, 2022).

Ectothermic organisms are highly susceptible to temperature changes because their body temperatures completely depend on environmental thermal conditions (Béltran *et al.*, 2021). In other words, temperature mediates all the physiological reactions of ectotherms, impacting their functional ecology (Angilletta, 2009). Thermal performance curves (TPCs) are commonly used to assess how body temperature affects performance and fitness in ectotherms, giving rise to predictions about how these organisms may be affected by climate change (Sinclair *et al.*, 2016). An organism's critical thermal limits are defined by the minimum temperature ( $CT_{min}$ ) and maximum temperature ( $CT_{max}$ ) it can withstand without losing motor coordination (Lutterschmidt and Hutchison, 1997). These limits are often incorporated into TPCs as benchmarks to improve understanding of relevant physiological limits and to frame the response of key biological traits. Special attention has been paid to  $CT_{max}$  because it can indicate the vulnerability of organisms to present and future temperature increases (Diamond *et al.*, 2012). However,  $CT_{max}$  can vary greatly among ectotherms and levels of biological organisation (i.e. individuals, populations, and communities; Verble-Pearson *et al.*, 2015; Nascimento *et al.*, 2022). Furthermore, this variation may be shaped by many biotic and abiotic factors (Sunday *et al.*, 2014; Leiva *et al.*, 2019).

Among ectotherms, ants stand out because they are ubiquitous and abundant in almost all terrestrial ecosystems, where they mediate many ecological processes and provide essential ecosystem services such as seed dispersal, protection against herbivores, and nutrient cycling (Del Toro *et al.*, 2012; Elizalde *et al.*, 2020). Because they are social insects, ants are an excellent

model for assessing differences in  $CT_{max}$  at different levels of biological organisation and for examining the factors behind these differences. Indeed, in ants,  $CT_{max}$  can be measured at the colony level (among nestmate workers), population level (among colonies of the same species), and community level (among different species) (Baudier and O'Donnell, 2020; Bujan *et al.*, 2020; O'Donnell *et al.*, 2020). That said, most research to date has focused on ant  $CT_{max}$  mainly at the community level; while some colony-level studies exist as well, population-level studies are scarce (Nascimento *et al.*, 2022). Also rare is work that simultaneously analyses differences in  $CT_{max}$  at all three levels (but see Verble-Pearson *et al.*, 2015). However, taking a more holistic approach is crucial in better understanding how species may be affected under future conditions of climate change (Roeder *et al.*, 2021). It is necessary to consider multiple organisational levels in tandem because ant colonies may have subclasses of workers that differ in body size and  $CT_{max}$  (Baudier and O'Donnell, 2020). Ant body size may also vary among colonies as a result of environmental factors (Shik *et al.*, 2019; Oliveira *et al.*, 2022), which can lead to variation in  $CT_{max}$  within species (Baudier and O'Donnell, 2020). While a few studies have looked at a small number of highly polymorphic species (i.e. those displaying marked within-colony variation in worker size), we do not know how much  $CT_{max}$  varies among workers across a broader range of polymorphic species or in monomorphic species (i.e. those displaying limited within-colony variation in worker size). At the colony level, thermal performance can be better assessed by using the temperature-dependent rates of key biological processes, such as metabolic rates (Shik *et al.*, 2019) or brood development rates (Penick *et al.*, 2017). While  $CT_{max}$  reflects just one aspect of performance, it is also an important thermal trait because it affects worker foraging and, consequently, colony energy supply (Arnan *et al.*, 2022). If selection pressure is greater on more thermally vulnerable workers, it will take a toll on colony fitness and persistence (Baudier and O'Donnell, 2017). In such cases, temperature increases could have negative effects at the colony level, an impact that would be obscured when exclusively analysing community-level responses.

Ant  $CT_{max}$  can differ due to many biotic and abiotic factors (Roeder *et al.*, 2021; Nascimento *et al.*, 2022). Studies have looked at how ant  $CT_{max}$  relates to microhabitat use (e.g. Baudier *et al.*, 2015, 2018; Kaspari *et al.*, 2015), evolutionary history (e.g. Diamond *et al.*, 2012; Arnan and Blüthgen, 2015), and body size (e.g. Kaspari *et al.*, 2015; Verble-Pearson *et al.*, 2015). However, the results of this research have been inconsistent. Furthermore, such work has rarely examined the combined effects of these factors and their ability to explain variation in ant  $CT_{max}$ . For example, microhabitat appears to have a strong effect on ant  $CT_{max}$  (Baudier *et al.*, 2015, 2018; Kaspari *et al.*, 2015; Bujan *et al.*, 2020) that could be underlain by species evolutionary history because microhabitat use is a highly conserved trait in ants (Lucky *et al.*, 2013). Thus, it is only possible to determine each factor's contribution if both are analysed in tandem.

Simultaneously exploring how different factors drive  $CT_{max}$  in ants is the foundation for understanding how ants will respond to future temperature increases, including which species may be more susceptible or possess greater adaptive potential (Nascimento *et al.*, 2022). Such information is especially critical for tropical regions, where ants already live close to their  $CT_{max}$  values and studies remain infrequent (Diamond and Chick, 2018). In the dry tropical forest of northeastern Brazil, for example, climate models predict a 3–6°C increase in temperature

by 2100 (Magrin *et al.*, 2014), which will likely expose the region's ant fauna to severe heat stress.

Given this context, our study aims to characterise  $CT_{max}$  in ants inhabiting a semiarid neotropical region and to understand the underlying factors (i.e. phylogeny, nesting microhabitat, and body size) operating at different levels of biological organisation – the community level (among species), the population level (among colonies within species), and the colony level (among workers within colonies). We addressed the following five questions: (1) How variable is  $CT_{max}$  within an ant community? (2) Are differences in community-level  $CT_{max}$  explained by species nesting microhabitat, body size, and/or evolutionary history? (3) Do colonies of the same species exhibit different  $CT_{max}$  values? (4) Do workers from monomorphic vs. polymorphic colonies exhibit different  $CT_{max}$  values? (5) Which level of organisation displays the greatest degree of variability in  $CT_{max}$ ?

## Materials and methods

### Study area

This study was conducted in the city of Garanhuns (Latitude: 8° 53'27" South, Longitude: 36°29'48" West), located in the rural region of Pernambuco, northeastern Brazil. The mean annual temperature is 20°C; temperatures decrease to a minimum of 15°C in the winter and increase to a maximum of 30°C in the summer. The climate is hot, tropical, subhumid, and dry (Barbosa *et al.*, 2016), a result of the city's location in a transitional zone between Zona da Mata and Sertão, where climates converge. The area is characterised by semideciduous seasonal vegetation, where there is ecological interplay with natural humid forests; phytogeographically, it is classified as Atlantic Forest and Caatinga (Costa *et al.*, 2014).

### Ant sampling

Ants were randomly sampled at several locations in Garanhuns from December 2020 to November 2021. We targeted tree trunks, soil, garbage, and lawns in city squares and parks. Sampling took place in the morning, in the afternoon, and at night. Carbohydrate- and protein-based baits were used to attract ants when necessary. The baits were solely used to attract the ants; none of the ants collected had actually consumed the bait. This measure was taken because carbohydrate consumption can increase ant heat tolerance (Freires *et al.*, 2023). Using an entomological aspirator, 20 workers were collected per colony for 3–4 colonies per species; these colonies were separated by at least 100 m. For a given species, colonies were sampled at the same time of day within a 4-month period to minimise the effects of seasonality. That said, for some species, particularly arboreal species, it was impossible to sample 20 workers. In such cases, we collected at least six individuals, which is the standard minimum sample size when characterising species traits (Gaudard *et al.*, 2019). To ensure that the workers belonged to the same colony, we first checked if the ants were following the same trail. After sampling the workers, we immediately placed them in 50-ml Falcon tubes; each tube contained a small cotton ball soaked in water to prevent ant desiccation. Within the tubes, the workers did not display aggressive behaviour towards each other, which reinforces the idea that they were nestmates. The workers were then identified to species or morphospecies using the ant collection at the laboratory of Ecology, Botany, and Ethnobiology, University of

Pernambuco (LEBE) –Garanhuns Campus. We also sent samples of all the ants collected to the Ant Biology and Systematics Laboratory (director: Dr Rodrigo M. Feitosa) at the Federal University of Paraná. An ant taxonomist confirmed the identities of the species and morphospecies. Hereafter, for simplicity's sake, we will use the term species to refer to both species and morphospecies.

### Maximum thermal tolerance assays

The workers we sampled were immediately taken to the laboratory to measure  $CT_{max}$ . No more than 4 h passed between worker collection and the beginning of the  $CT_{max}$  measurements; the minimum time elapsed was 1.5 h. In the laboratory, workers were first transferred from Falcon tubes to 7-l plastic trays. Next, the ants were placed in 1.5-ml microcentrifuge tubes (1 worker per tube) plugged with cotton, preventing access to any thermal refuges (Oberg *et al.*, 2012). Workers that were visibly injured (e.g. limping or moving very slowly) were removed and discarded. Each tube was placed in a randomly chosen locule of a dry bath heater (8×6 Thermal-Lok Dry Heat Bath, USA Scientific, Orlando, Florida) that had been preheated to 38°C. Heating block temperature was increased by 1°C every 3 min (Arnan *et al.*, 2022). We used 38°C as the starting temperature because, in preliminary analyses, no species had a  $CT_{max}$  lower than this value. Although the rate of increase could affect absolute  $CT_{max}$  (Roeder *et al.*, 2021), such is not a concern given our interest in comparing workers experiencing the same protocol. At the end of each 3-min period, we checked levels of ant movement, observing whether each ant could reorient itself after being disturbed by us lightly tapping on the tube. During testing, at least three individuals from each colony were kept in tubes outside of the device as controls to observe whether any of these individuals died from stress. No mortality was observed. The temperature at which an ant lost muscle coordination was defined as its  $CT_{max}$  (Diamond *et al.*, 2012).

### Body size

We measured mesosoma length (i.e. Weber's length) for all the ants used in the  $CT_{max}$  assays. This metric serves as a proxy of total body size. Measurements took place using a 1-mm paper placed over the stage of a dissecting microscope. The ants were placed in profile on the graph paper, and their legs were stretched out, so that we could clearly see the point where the pronotum met the cervical shield. We measured the distance between that point and the posterior basal angle of the metapleuron.

### Nesting microhabitat

We identified the nesting microhabitat used by each of the 47 species we had sampled utilising the literature, field observations, and consultations with experts. Ants were classified as either ground-nesting species or tree-nesting species.

### Phylogenetic history

We tested for the presence of a phylogenetic signal in  $CT_{max}$  by employing a time- and genus-calibrated ant phylogeny (Moreau and Bell, 2013). This phylogeny was then pruned to retain a single species per genus and thus generate a genus-level phylogeny. Four genera represented within our samples (*Holcoponera*,

*Mycetomoellerius*, *Mycocepurus*, and *Nylanderia*) were not present in the original phylogeny and were thus added as sibling species to their closest relatives: *Holcoponera* with *Gnamptogenys* (Camacho *et al.*, 2022), *Mycetomoellerius* with *Sericomyrmex* (Hanisch *et al.*, 2022), *Mycocepurus* with *Myrmicocrypta* (Hanisch *et al.*, 2022), and *Nylanderia* with *Paratrechina* (Ward *et al.*, 2016). Then, by manually editing the NEWICK tree, the species represented in our samples were inserted into the tree as polytomies at the basal genus level (Supplementary fig. S1). Next, we used Blomberg's K (Blomberg *et al.*, 2003) and Pagel's  $\lambda$  (Pagel, 1999) tests to quantify the phylogenetic signal in  $CT_{max}$ .

### Statistical analyses

One of our goals was to compare variation in  $CT_{max}$  at different levels of biological organisation. We thus graphed all the colony-level data using boxplots to identify and exclude potential outliers that could lead us to spurious results. Several colonies showed outliers, which were always on the low side of values (i.e. with very low heat tolerance), suggesting that they might be somehow weakened individuals. Our approach was to eliminate data (35) for workers whose  $CT_{max}$  deviated more than 3°C from the mean  $CT_{max}$  of colonies that had outliers.

Interspecific differences in  $CT_{max}$  were analysed using a general linear mixed model (GLMM), where the response variable was  $CT_{max}$ , the fixed factor was species, and the random factors were colony within species. The individual workers were the replicates ( $n = 1864$ ). Data were checked for normality and homoscedasticity.

To identify the factors that best explained differences in species-level  $CT_{max}$ , and since we detected a phylogenetic signal in  $CT_{max}$  (see results), we ran a phylogenetic generalised least squares (PGLS) model utilising a variance-covariance matrix structured by the species' inferred phylogenetic relationships. The response variable was  $CT_{max}$  (species-specific mean), and the fixed factors were body size (species-specific mean) and nesting microhabitat. The species sampled were the replicates ( $n = 47$ ).

To test whether  $CT_{max}$  differed among colonies within species, GLMs were used; colony-specific  $CT_{max}$  was the response variable, and colony was the fixed factor. One model was conducted for each species, for which three or four colonies had been sampled (total of 16 models). Mean body size can vary intraspecifically among colonies and could thus explain colony-level differences in  $CT_{max}$ . Consequently, the models were re-run after adding mean colony-level body size as a covariate.

To test whether there was a relationship between worker size and  $CT_{max}$  within colonies, we performed regression models where linear and quadratic terms for worker body size were the explanatory variables and worker  $CT_{max}$  was the response variable. We ran one model per colony. When the quadratic term was not significant, we reran the model without it.

To determine which level of biological organisation displayed the most variability in  $CT_{max}$ , we calculated two indices. First, we determined the range of  $CT_{max}$  at each level. At the community level, this metric was the difference between maximum and minimum  $CT_{max}$  across individuals. At the population level, it was the difference between maximum and minimum  $CT_{max}$  across individuals within species. At the colony level, it was the difference between maximum and minimum  $CT_{max}$  across individuals within colonies. Second, we calculated the mean and standard deviation of  $CT_{max}$  for each level; the standard deviation was then divided by the mean to obtain the level's coefficient of

variation (CV). Using the CV, we determined how much each level varied in °C using mean  $CT_{max}$  for the community, a given species population, and a given colony as the standards of reference (i.e. for each level, we multiplied mean  $CT_{max}$  by CV to obtain variation in °C). For the community level, the standard of reference was community mean  $CT_{max}$ . For the population level, the two standards of reference were community mean  $CT_{max}$  and population mean  $CT_{max}$  (for the 16 species for which we had population-level data). For the colony level, there were three standards of reference: community mean  $CT_{max}$ , population mean  $CT_{max}$ , and colony mean  $CT_{max}$ .

All the analyses were performed using R software (R Development Core Team, 2019). The GLMs were performed using the `glm` function in the `stats` package; the GLMMs were performed with the `lme` function in the `nlme` package (Pinheiro et al., 2021); and the PGLS were performed with the `pgls` function in the `caper` package (Orme et al., 2013).

## Results

We collected  $CT_{max}$  data for 1864 workers from 99 colonies. Altogether, 47 species belonging to 6 subfamilies and 26 genera were sampled (table S1). The best represented subfamilies were Myrmicinae (23 species) and Formicidae (10 species) followed by Pseudomyrmecinae (6 species), Dolichoderinae (4 species), Ectatomminae (2 species), and Ponerinae (2 species). There were 29 ground-nesting species and 18 tree-nesting species. Species-specific body size varied, ranging from 0.4 to 3.43 mm in mesosome length (Supplementary table S1).

### Community-level $CT_{max}$

Mean  $CT_{max}$  differed significantly among the 47 ant species (GLMM:  $\chi^2_{46} = 295.1$ ,  $p < 0.001$ ). Two ground-nesting, fungus-growing ants displayed the lowest values (mean  $\pm$  SD):  $39.3^\circ\text{C} \pm 1.3$  for *Mycocetopus smithii* and  $40.6^\circ\text{C} \pm 0.6$  for *Sericomyrmex mayr* (fig. 1). Two arboreal species displayed the highest values:  $49.7^\circ\text{C} \pm 0.5$  for *Pseudomyrmex termitarius* and  $48.9^\circ\text{C} \pm 0.4$  for *Camponotus blandus* (fig. 1; Supplementary table S1). Mean  $CT_{max}$  ( $\pm$  SD) for the community was  $44.8 \pm 1.7^\circ\text{C}$ .

### Ability of nesting microhabitat, phylogeny, and body size to explain species $CT_{max}$

We detected a phylogenetic signal in  $CT_{max}$  based on both Blomberg's  $K$  ( $K = 0.002$ ,  $p = 0.001$ ) and Pagel's  $\lambda$  ( $\lambda = 0.77$ ,  $p = 0.003$ ). More specifically, more closely related species had more similar  $CT_{max}$  values than did less closely related species (Supplementary fig. S1).

Only body size had a significant effect on species  $CT_{max}$ , decreasing with increasing body size (Supplementary fig. S1; table 1).

### Population-level $CT_{max}$

Nine of the 16 species displayed significant differences in mean  $CT_{max}$  among colonies (fig. 2; Supplementary table S2). When body size was included as a covariate, the results were generally similar, except that *Pheidole* sp.1 no longer exhibited a significant difference in among-colony  $CT_{max}$  but *Tetramorium simillimum* did. This result suggests that, within species, differences in  $CT_{max}$  among colonies were poorly explained by colony-specific differences in worker size.

### Colony-level $CT_{max}$

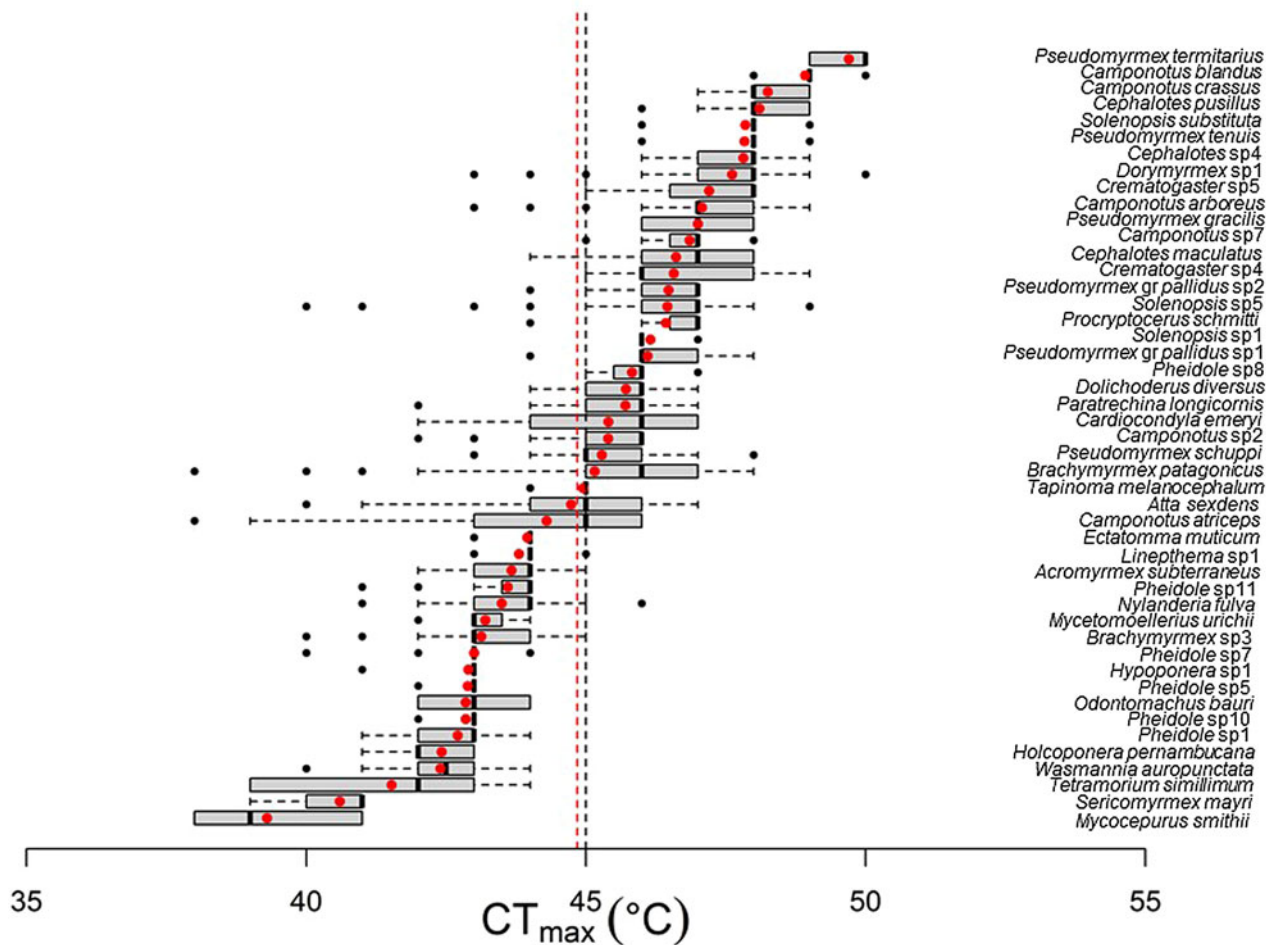
Nestmate workers typically exhibited different  $CT_{max}$  values (figs 3 and 4), as observed in 92 colonies ( $n = 99$  colonies across 47 species; Supplementary table S3). In one colony, workers even had differences of up to  $9^\circ\text{C}$  (*Solenopsis* sp.5). In a few other colonies,  $CT_{max}$  was the same for all workers (*Acromyrmex subterraneus*, *C. blandus*, *Crematogaster* sp.4, *Dolichoderus diversus*, *Holcoperona pernambucana*, and *Odontomachus bauri*). Interestingly, colonies of monomorphic species (e.g. *Nylanderia fulva* and *Brachymyrmex patagonicus*) could vary as much in  $CT_{max}$  as colonies of polymorphic species (e.g. *Atta sexdens* and *A. subterraneus*) (figs 3 and 4, Supplementary table S3). For polymorphic species, worker  $CT_{max}$  was positively related to body size in some colonies, notably in three colonies of *A. sexdens* and one colony of *A. subterraneus* (fig. 3; Supplementary table S3); for the *A. subterraneus* colony and one of the *A. sexdens*' colonies, there was a positive linear relationship. For the other two *A. sexdens* colonies, the relationship was quadratic:  $CT_{max}$  increased with body size only up to a certain point. In summary, in some polymorphic colonies, workers displayed variable  $CT_{max}$  values that were positively correlated with body size. In other polymorphic colonies, workers displayed variable  $CT_{max}$  values that did not correlate with body size. In yet other polymorphic colonies, there was no variability in  $CT_{max}$ . Finally, in monomorphic colonies, workers either did or did not display variability in  $CT_{max}$  (fig. 4).

### Variability in $CT_{max}$ at different levels of biological organisation

At the community level, the range of  $CT_{max}$  was  $12^\circ\text{C}$  ( $38\text{--}50^\circ\text{C}$ ). The CV was 3.8%, which means variation was as high as  $1.7^\circ\text{C}$ . At the population level, *B. patagonicus* had the largest range ( $10^\circ\text{C}$ ;  $38\text{--}48^\circ\text{C}$ ), while *O. bauri* had the smallest range ( $1^\circ\text{C}$ ;  $42\text{--}43^\circ\text{C}$ ). The mean range ( $\pm$ SD) of  $CT_{max}$  for colonies of the same species was  $5.3 \pm 2.4^\circ\text{C}$  across all the species sampled. *Brachymyrmex patagonicus* had a CV of 5.7%, which means variation was as high as  $2.5^\circ\text{C}$  when using community and population mean  $CT_{max}$  (both  $45^\circ\text{C}$ ) as standards of reference. *Odontomachus bauri* had a CV of 0.8%, which means variation was as high as  $0.4$  or  $0.3^\circ\text{C}$  when using community mean  $CT_{max}$  ( $45^\circ\text{C}$ ) or population mean  $CT_{max}$  ( $43^\circ\text{C}$ ) as the standard of reference, respectively. At the colony level, the greatest variability was seen among workers of *Solenopsis* sp.5, for which the range of  $CT_{max}$  was  $9^\circ\text{C}$  ( $40\text{--}49^\circ\text{C}$ ). In contrast, no variability was observed among workers of single colonies of *A. subterraneus*, *C. blandus*, *Crematogaster* sp.4, *D. diversus*, and *H. pernambucana* or of three colonies of *O. bauri*. The mean ( $\pm$ SD) range of  $CT_{max}$  among workers of the same colony across all colonies was  $2.7 \pm 1.8^\circ\text{C}$ . The highest colony-level CV (7.4%) was seen in a colony of *Solenopsis* sp. 5. Thus, variation was as high as  $3.3$  or  $3.4^\circ\text{C}$  when community mean  $CT_{max}$  ( $45^\circ\text{C}$ ) or population mean  $CT_{max}$  ( $46^\circ\text{C}$ ) was the standard of reference, respectively. It was as high as  $3.3^\circ\text{C}$  when colony mean  $CT_{max}$  ( $45^\circ\text{C}$ ) was the standard of reference.

## Discussion

First, we examined community-level variability in  $CT_{max}$ . The difference between maximum and minimum species-specific  $CT_{max}$  was  $12^\circ\text{C}$  ( $n = 47$  species), which is much lower than what has been found in other studies in tropical regions (Kaspari et al., 2015; Nowrouzi et al., 2018). For example, Kaspari et al. (2015)



**Figure 1.** Boxplots (horizontal bars) showing the species-level medians, means (red dots), and ranges of  $CT_{max}$  for the 47 ant species. The median and mean  $CT_{max}$  of the ant community are indicated by black and red dashed lines, respectively. Species were ordered by ascending mean  $CT_{max}$ .

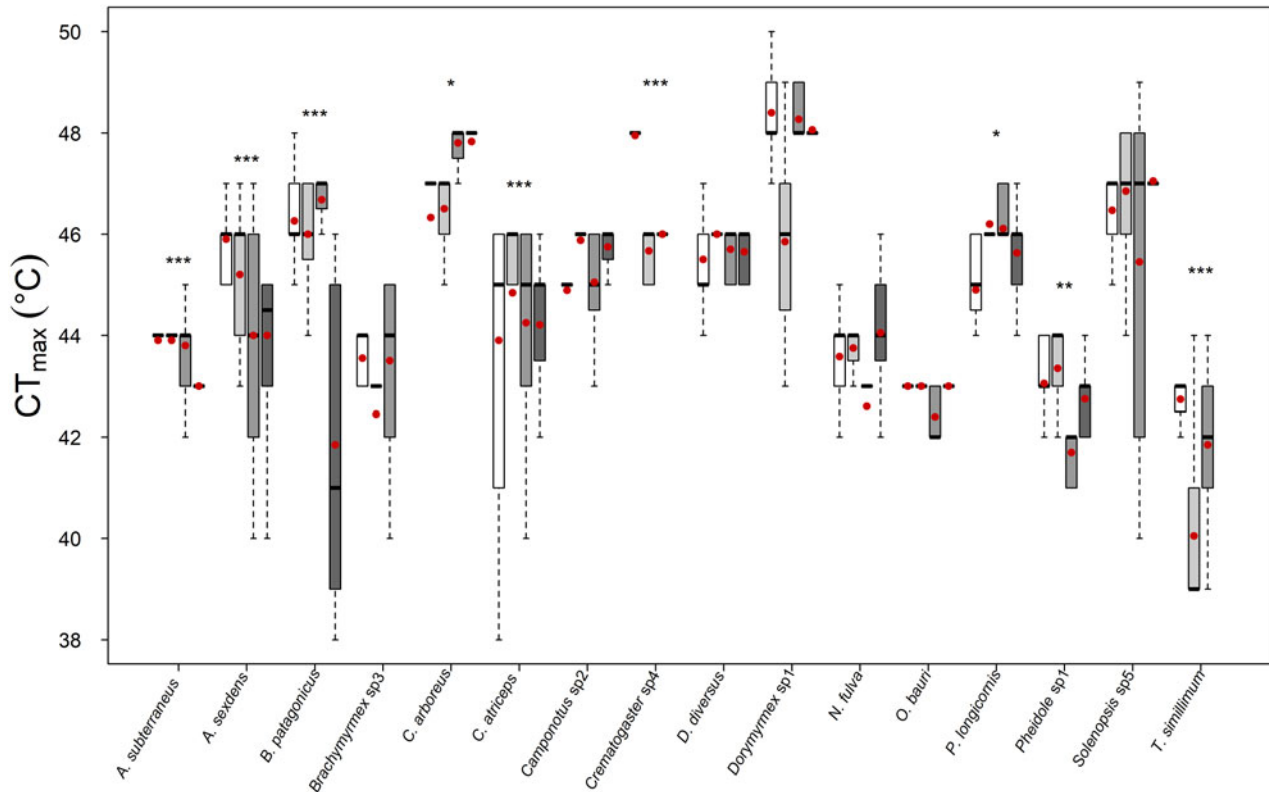
observed that the range of  $CT_{max}$  was 15°C for a rainforest ant community in Panama ( $n = 88$  species). Australian rainforest ant communities ( $n = 20$  species) had a  $CT_{max}$  range of 28°C (Nowrouzi *et al.*, 2018). Similar results have been seen in temperate regions. For instance, for North American ants in different ecosystems ( $n = 132$  species), Bujan *et al.* (2020) observed that the range of  $CT_{max}$  was 18.2°C. However, in a temperate ecosystem (Harvard Forest), Oberg *et al.* (2012) found that the ant community ( $n = 16$  species) had a much lower value: 8°C. These results show that  $CT_{max}$  range at the community scale can vary greatly across biogeographical regions, within which there exists habitat-related heterogeneity. Understanding these dynamics is particularly important because communities containing species with a broader range of  $CT_{max}$  values may be more resilient in the face of temperature changes (Arnan *et al.*, 2015).

The interspecific variation in  $CT_{max}$  that we observed also suggests that climate change, especially global warming, will affect species differently (Roeder *et al.*, 2021; Nascimento *et al.*, 2022).

Second, we explored whether three key factors explained  $CT_{max}$  within the ant community. We found that phylogeny and body size, but not nesting microhabitat, explained differences in  $CT_{max}$  among species. Our results underscore that species-specific  $CT_{max}$  is highly conserved in ants. Such has been seen in studies at larger spatial scales (Diamond *et al.*, 2012; Diamond and Chick, 2018), but our study shows that the same is true at smaller spatial scales. In the context of climate change, these results are concerning because they suggest that the ants in our study region may be limited in their capacity to increase  $CT_{max}$  because of phylogenetic constraints (Diamond and Chick, 2018). With regards to body size, we found a negative relationship between  $CT_{max}$  and species body size: larger ants had lower  $CT_{max}$  values. This negative relationship has been observed in other studies (Verble-Pearson *et al.*, 2015). Indeed, a recent review found that  $CT_{max}$  and ant body size are inconsistently related on small spatial scales – their association can be positive, negative, or non-existent (Roeder *et al.*, 2021; Nascimento *et al.*, 2022). Our results could have been influenced by the fact that we conducted our study in an urban area, where large ants may be less common as a consequence of anthropogenic pressures

**Table 1.** Results of the phylogenetic generalised least square models exploring the effects of worker body size (mesosoma length) and nesting microhabitat on species-specific  $CT_{max}$

	Df	Sum Sq	Mean Sq	F value	P-value
Body size	1	31.96	31.96	4.28	0.044
Nesting microhabitat	1	4.84	4.83	0.65	0.425

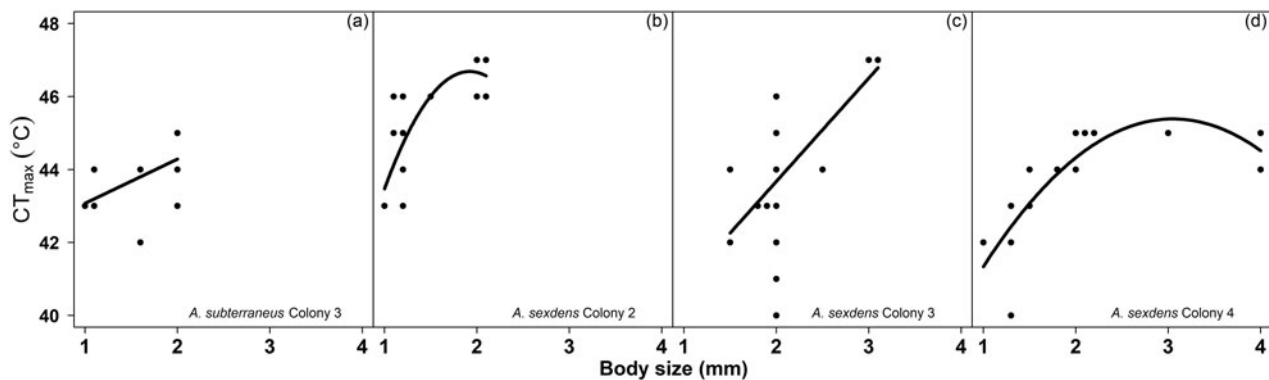


**Figure 2.** Box plots showing the colony-level medians, means (red dots), and ranges of  $CT_{max}$  for the 16 ant species ( $n=3$  or 4 colonies sampled) in the analysis. Significance values: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; and \*\*\*,  $P < 0.001$ . Each colour represents one colony per species. Abbreviations: *A. subterraneus*, *Acromyrmex subterraneus*; *A. sexdens*, *Atta sexdens*; *B. patagonicus*, *Brachymyrmex patagonicus*; *C. arboreus*, *Camponotus arboreus*; *C. atriceps*, *Camponotus atriceps*; *D. diversus*, *Dolichoderus diversus*; *N. fulva*, *Nylanderia fulva*; *O. bauri*, *Odontomachus bauri*; *P. longicornis*, *Paratrechina longicornis*; *T. simillimum*, *Tetramorium simillimum*.

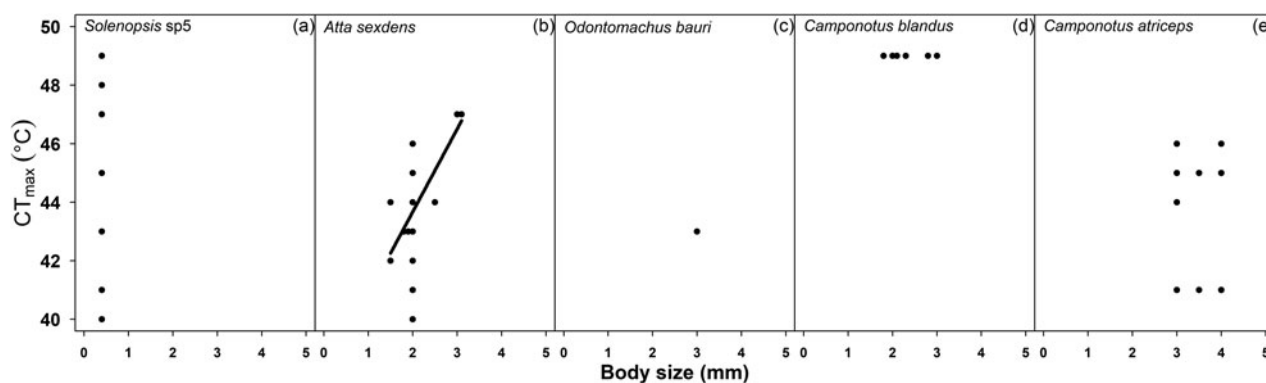
(Gibb *et al.*, 2015). In addition, the ecological characteristics of the species studied may also have played a role (Verble-Pearson *et al.*, 2015). For example, two of the larger species – *Camponotus atriceps* and *Camponotus sp.2* – start foraging at nightfall, and nocturnal ants appear to have lower  $CT_{max}$  values (Esch *et al.*, 2017). To understand how body size may interact with thermal tolerance in ants, more studies are needed, notably those comparing how this relationship manifests itself under different environmental conditions and/or across varying pools of species that differ in biogeographical origin or evolutionary history

(Nascimento *et al.*, 2022). Finally, contrary to previous studies that observed higher  $CT_{max}$  values in tree-nesting ants (Kaspari *et al.*, 2015; Bujan *et al.*, 2020), our study found no significant differences between ground-nesting and tree-nesting species. Microhabitat use is a highly conserved trait in Formicidae (Lucky *et al.*, 2013). Given that our analysis controls for phylogenetic relatedness, this effect may have disappeared.

Third, within species, colonies displayed different  $CT_{max}$  values (9 of the 16 species studied); body size had no effect. Although we only tested 3–4 colonies per species, this was enough



**Figure 3.** Relationship between worker body size (mesosoma length) and  $CT_{max}$  for the four colonies with significant regression results. The number of workers per colony was 20. Each black dot represents a worker. Some workers overlapped in  $CT_{max}$  and/or body size. Abbreviation: *A. subterraneus*, *Acromyrmex subterraneus*; *A. sexdens*, *Atta sexdens*.



**Figure 4.** Within-colony variability in worker body size (mesosoma length) and  $CT_{max}$  for five species that displayed different relationships between these two variables: (A) monomorphic colony of *Solenopsis* sp.5 with highly variable in  $CT_{max}$ ; (B) polymorphic colony of *Atta sexdens* in which body size and  $CT_{max}$  were positively correlated; (C) monomorphic colony of *Odontomachus bauri* with no variability in  $CT_{max}$ ; (D) polymorphic colony of *Camponotus blandus* with variability in body size but not in  $CT_{max}$ ; and (E) polymorphic colony of *Camponotus atriceps* with variability in both body size and  $CT_{max}$  without the variables being correlated. The number of workers per colony was 20. Each black dot represents one worker. Some workers overlapped in  $CT_{max}$  and/or body size.

to find differences in  $CT_{max}$  within species. In previous research, colony-level differences in  $CT_{max}$  were observed for *Messor arenarius* ( $n = 3$  colonies) and *M. ebeninus* ( $n = 5$  colonies) (O'Donnell *et al.*, 2020). In contrast, they were not seen in *Pogonomyrmex barbatus* ( $n = 10$  colonies) (Roeder *et al.*, 2022). All three species are polymorphic or variable in size. For *M. arenarius* and *M. ebeninus*, colony-level differences in  $CT_{max}$  were also unrelated to worker size (O'Donnell *et al.*, 2020). There are a few hypotheses that could explain differences in  $CT_{max}$  among colonies. First, body size can affect  $CT_{max}$  at the individual level, and, consequently, this relationship may scale up to the colony level (Cerdá and Retana, 2000). Second, diet may play a role: access to higher levels of sucrose might increase worker  $CT_{max}$  (Bujan and Kaspari, 2017; Freires *et al.*, 2023). Third, ants can display phenotypic plasticity in response to the environmental temperatures they experience (Nascimento *et al.*, 2022), such that colonies of the same species can exhibit different  $CT_{max}$  values if they live under different microclimatic conditions. The first hypothesis seems to have little support; the second has not yet been tested; and the third is generally supported by past research (Nascimento *et al.*, 2022). It is paramount that future work focus on the effects of diet and plasticity on ant  $CT_{max}$  among colonies within species (Roeder *et al.*, 2021; Nascimento *et al.*, 2022). Although past research has indicated that ectotherms seem unable to greatly increase  $CT_{max}$  (Bennett *et al.*, 2021; Pottier *et al.*, 2022), ants may be an exception. Our study showed that, among colonies within species, differences in mean  $CT_{max}$  could exceed 4°C.

Fourth, we discovered that worker  $CT_{max}$  was not influenced by the degree of colony polymorphism. Within colonies,  $CT_{max}$  could vary tremendously (e.g. up to 9°C) in a way that was not always associated with worker size. Indeed, the variability in  $CT_{max}$  within colonies was nearly equal to the variability within the community. Furthermore, some polymorphic colonies showed limited variability in  $CT_{max}$ , while some monomorphic colonies showed marked variability in  $CT_{max}$ . We observed a pronounced positive relationship between body size and  $CT_{max}$  in the polymorphic species *A. sexdens* ( $n = 3$  colonies) and *A. subterraneus* ( $n = 1$  colony). However, no such pattern was seen in the colonies of other polymorphic species (i.e. *Mycetomoellerius urichii* and *Camponotus* spp.) or in those of less polymorphic species (i.e. *Pheidole* spp.). Previous research analysing the effect of body

size on within-colony  $CT_{max}$  has yielded two contrasting results: (1) larger workers had higher  $CT_{max}$  than smaller workers (Cerdá and Retana, 2000; Baudier *et al.*, 2015) and (2) larger and smaller workers had similar  $CT_{max}$  (Lytle *et al.*, 2020; Yela *et al.*, 2020). A positive relationship between worker size and  $CT_{max}$  has mainly been found in highly polymorphic species, such as those in the genus *Atta* (Ribeiro *et al.*, 2012; Baudier and O'Donnell, 2020), those in the genus *Cataglyphis* (Cerdá and Retana, 2000), or those in army ant taxa (Baudier *et al.*, 2015, 2018). The absence of a relationship between worker size and  $CT_{max}$  has been seen in species with a low degree of polymorphism, such as *Ectatomma ruidum* (Esch *et al.*, 2017), *Solenopsis invicta* (Lytle *et al.*, 2020), and *Temnothorax curvispinosus* (Yilmaz *et al.*, 2019). In contrast to the above, there is little research looking at variation in  $CT_{max}$  among workers in monomorphic ants, which could possibly be explained by stress, age, or nutritional status (Nyamukondiwa and Terblanche, 2009). Regardless of the degree of morphological variation among workers, the presence of workers with different  $CT_{max}$  values allows colonies to explore microhabitats with different temperatures.

Fifth, our results showed that the greatest variability in  $CT_{max}$  existed at the community level (based on the  $CT_{max}$  ranges) and colony level (based on the CV for each level). Only one previous study had examined variability in ant  $CT_{max}$  at all three levels of biological organisation, and it found that variability was greatest at the community level (Verble-Pearson *et al.*, 2015). Such is not uncommon. For instance, the variation in  $CT_{max}$  seen in an ant community in Panama (Kaspari *et al.*, 2015) accounted for 74% of the variance in a global data set that included 269 ant populations found from 0 to 66° North in latitude (Diamond *et al.*, 2012). Here, we also found dramatic variation at the community level (albeit less than in Kaspari *et al.*, 2015). However, it is worth mentioning that a single colony or population can display almost as much variation as an entire community (40–49 and 38–48°C compared to 38–50°C, respectively). It is unlikely that our colony- and population-level results were influenced by the number of workers sampled, given that sample sizes were largest for the species with the highest and lowest degree of variability. However, it could be that our study area – an urban environment – mainly contains thermal generalist species and that colony-level values thus more closely reflect community values (Franzén *et al.*, 2020). Furthermore, genetic variation

among populations can also explain variation in  $CT_{max}$  within and among colonies (reviewed in Perez and Aron, 2020). Given the limited ability of morphology to explain the striking variability that we observed, future work must elucidate the underlying factors at play and identify the species that have a greater capacity to increase  $CT_{max}$  in response to climate change.

## Conclusion

Neotropical species in northeastern Brazil display differences in  $CT_{max}$  of up to  $\sim 12^\circ\text{C}$ . Species-specific  $CT_{max}$  is best explained by phylogeny, which could clearly constrain the ability of ants to deal with climate change. However, ant colonies can often cope with challenging temperatures via acclimatisation, thermal plasticity and/or improved nutrition (Nascimento *et al.*, 2022; Freires *et al.*, 2023), which should allow them to navigate global temperature increases. Body size also explained differences in  $CT_{max}$  among species but not among colonies within species. That said, a positive correlation did exist in some colonies of highly polymorphic species; it may play an important role in colony division of labour and in the context of climate change. The presence of differences in colony-level  $CT_{max}$  within species, whether monomorphic or polymorphic, demonstrates that ants possess the ability to adapt to different thermal conditions, although this issue has rarely been explored. We wish to emphasise that we observed at least as much variability in  $CT_{max}$  within colonies as within the community, a fact that could help shape the adaptive potential of ant populations as they face changes in environmental temperature. It is urgent to conduct new research that clarifies the factors affecting variability in  $CT_{max}$  among and within colonies.

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