Variation in thermal tolerance of North American ants

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ABSTRACT

Changing climates are predicted to alter the distribution of thermal niches. Small ectotherms such as ants may be particularly vulnerable to heat injury and death. We quantified the critical thermal maxima of 92 ant colonies representing 14 common temperate ant species. The mean CT_{max} for all measured ants was 47.8 °C ($\pm$ 0.27; range=40.2–51.2 °C), and within-colony variation was lower than among-colony variation. Critical thermal maxima differed among species and were negatively correlated with body size. Results of this study illustrate the importance of accounting for mass, among and within colony variation, and interspecific differences in diel activity patterns, which are often neglected in studies of ant thermal physiology.

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1. Introduction

Climate change is altering the thermal profiles of habitats currently occupied by many plant and animal species (Sinervo et al., 2010; Diamond et al., 2012). Shifts in average temperatures have already narrowed the ranges of some species (Parmesan, 2006) while increasing the ranges of others (e.g., Carroll et al., 2003; Jepsen et al., 2008; Walther et al., 2002). Animals inhabiting different microhabitats or geographic regions may not experience equal magnitudes of temperature change (Coulson et al., 1993). For example, temperate ants, the focus of this study, appear to be less susceptible to warming than tropical ants, and canopy ants appear more vulnerable than leaf litter ants (Huey and Tewksbury, 2009; Wittman et al., 2010; Diamond et al., 2012). At smaller scales, differential thermal preferences among species may be one way in which species subdivide niche space. Specifically, subordinant ants may benefit from foraging at a broader range of temperatures than dominant ants (Cerda et al., 1998; Bestelmeyer, 2000; Lessard et al., 2009). Thus, local ant diversity may be maintained in part by thermal niche diversity mediated by behavior and body size (Lessard et al., 2009; Pelini et al., 2011, Oberg et al., 2012).

As ectotherms, ants and other insects are especially vulnerable to injury and death from extreme temperatures (Denlinger and Yocum,

1998). The lethality of heat exposure is a function of an insect's physiological response, body size, food status, evolutionary history, and extrinsic parameters such as ambient humidity, and the magnitude, rate, and duration of temperature increase (Cerda et al., 1998; Terblanche et al., 2007; Chown et al., 2009; Santos et al., 2011; Oberg et al., 2012; Overgaard et al., 2012; Ribeiro et al., 2012).

Most insects do not maintain normal behavioral and physiological functions at temperatures > 50 °C (Neven, 2000), but thermophily and extreme heat tolerance occur in some species. For example, firebrats (Thermobia domestica) survive for prolonged periods at temperatures up to 55°°C (Sweetman, 1938; Edwards and Nutting, 1950). Additionally, the desert ant Cataglyphis bombycina exclusively forages when surface temperatures are above 60 °C (Wehner et al., 1992). The Australian ant Melophorus bagoti also avoids cool temperatures and forages when soil temperatures are above 70 °C (Christian and Morton, 1992). Apart from these extreme examples of heat tolerance, few studies provide basic measurements of thermal physiology (e.g., resting metabolic rate, thermal maxima, thermal minima) among ants within a region using standardized methods (but see Lighton and Turner, 2004; Diamond et al., 2012; Oberg et al., 2012; Kaspari et al., 2014).

As temperatures increase, insects exhibit a set of predictable behavioral responses, including spontaneous hyperactivity, loss of coordination and equilibrium, and finally quiescence (Friedlander et al., 1976). Such behavioral effects are sufficiently consistent that the cues most often used to measure insect physiological responses to heat are the loss of "righting reflex" (Huey et al., 1992)

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and onset of spasms (Lutterschmidt and Hutchison, 1997). Several studies have demonstrated the importance of using standardized ramping times and acclimation temperatures in studies of thermal limits (Terblanche et al., 2007; Chown et al., 2009; Santos et al., 2011; Overgaard et al., 2012). Slow rates of temperature increase are associated with lower critical thermal maxima in ants (Chown et al., 2009; Ribeiro et al., 2012), tsetse flies (Terblanche et al., 2007), and fruit flies (Overgaard et al., 2012), whereas faster rates of temperature increase are associated with higher critical thermal maxima.

We hypothesized that common temperate ant species differ in their ability to tolerate extreme temperatures and that these differences in thermal tolerance are related to body size. Based on similar studies of tropical and desert ants (e.g., Clemencet et al., 2010; Kaspari et al. 2014), we predicted that larger ants will have higher critical thermal maxima than smaller ants, and that differences within species and among colonies will be small relative to differences among species.

2. Materials and methods

2.1. Ant collection

Ants were collected from May 2010 through August 2013 from multiple locations in Arkansas and Texas, mostly within 30 km of Little Rock (34.74°N, 92.33°W) or Lubbock (33.59°N, 101.89°W), respectively. Collection efforts focused on common soil nesting, log/twig nesting, and arboreal ants. At least ten workers for each species were collected when it was not possible to collect entire colonies (Fig. 1). Ants were collected between the hours of 8:00 and 18:00 at temperatures ranging from 14 to 37 °C.

2.2. Thermal trials

Colonies were maintained in the laboratory at 23 °C for 12–72 h before measurements of thermal physiology were performed. We housed ants in plastic containers with water, ad libitum honey and tuna, and their natural nest substrate, when possible. During each trial, five monomorphic ants were randomly selected from a colony and

Fig. 1. Average (± SE) ant CT_{max} (C) and mass (mg) of the focal species. Numbers on the figure are ant species identifiers. 1=*Aphaenogaster fulva*, 2=*A. texana*, 3=*Camponotus pennsylvanicus*, 4=*Crematogaster lineolata*, 5=*C. obscurata*, 6=*Dorymyrmex flavus*, 7=*Formica incerta*, 8=*Linepithema humile*, 9=*Monomorium minimum*, 10=*Paratrechina longicornis*, 11,=*Pheidole dentigula*, 12=*Pogonomyrmex rugosus*, 13=*Solenopsis invicta*, 14=*Stenamma brevicorne*. Error bars are standard errors associated with average mass (horizontal) and CT_{max} (vertical) for each species. Analyses were run with and without circled (outlier) data points.

housed in a single vial. Ants from five colonies were observed during each trial. Thin plastic 10 dram (29 mmx88 mm) vials were submerged ca. 10 cm apart in a water bath with an initial temperature of 28 °C. The temperature of the water bath was increased at a constant rate (0.5 °C per min) until all ants within vials reached their thermal tolerance limits (CT_{max}). This limit was indicated by loss of the righting reflex (LORR; Huey et al., 1992). A HOBO data logger and probe (U23 Pro, Onset Computer Corporation) was used to confirm that the temperatures experienced in the vials were equal to the water bath temperature. As soon as LORR occurred, ants were removed from the hot water bath. A subsample of *Crematogaster lineolata* workers ($n=5$ colonies) was exposed to a second trial < 1 h after the first trial to examine the potential for heat hardening. Body size (dry mass; 48 h at 55 °C) was measured for ten similarly sized (same caste) workers of each species tested. Very small ants were weighed in groups and individual mass estimated by division.

2.3. Data analysis

We conducted all analyses on log_{10}-transformed data. We used analysis of covariance (ANCOVA) to compare thermal tolerances among variable numbers of replicate colonies per species with mass as the covariate (SAS 2009). *Crematogaster lineolata* heat-hardening data were analyzed with a paired *t*-test. When examining evolutionary patterns of phenotypic variation among species, it is important to account for non-independence of species traits in the analysis. We used an ultrametric phylogeny (Moreau and Bell, 2013) as the basis for a phylogenetic generalized least squares analysis (PGLS). In this analysis, we estimated Pagel's lambda (Pagel, 1999), a maximum likelihood estimate of the phylogenetic signal in the data, as part of this analysis. The PGLS analysis was run a second time excluding outliers (see below). All ants were identified to species using published keys, and voucher specimens were confirmed by taxonomists. Voucher specimens were deposited in the Watson Museum of Entomology at the University of Arkansas at Little Rock.

3. Results

We determined the critical thermal maximum (CT_{max}) for groups of worker ants ($n=5$ per group) from 92 colonies representing 14 species. The average (± SE) CT_{max} for all measured ants was 47.8 °C (± 0.27; range=40.2–51.2 °C, Fig. 2). Within-colony variation (average ± SE difference among individuals= 0.52 ± 0.04 °C, range=0.1–1.1 °C) was lower than among-colony variation (average ± SE difference among colonies=3.5 ± 0.60 °C, range=0.3–7.5 °C). The harvester ants (*Pogonomyrmex*) exhibited an extremely high CT_{max} relative to their mass (49.57 ± 0.45 °C). In addition, our sample only included a single colony for *Formica*. Thus, we conducted quantitative analyses both with and without these taxa included.

The estimated lambda value for thePGLS was zero in all tests, suggesting a lack of phylogenetic structure in the data. Under these circumstances, the coefficient of determination from the PGLS analysis is equivalent to the value estimated from a conventional regression analysis (see below). For the fourteen species used in this study, CT_{max} declined significantly and linearly with increasing mass (phylogenetic: $R^2=0.324$, $P=0.025$; non-phylogenetic: $R^2=0.324$, $P=0.042$). The relationship was stronger when *Pogonomyrmex* and *Formica incerta* were excluded (phylogenetic: $R^2=0.608$, $P=0.0009$, non-phylogenetic: $R^2=0.608$, $P=0.028$). The relationship between CT_{max} and mass did not differ among species (ANCOVA interaction $P=0.44$), but critical thermal maxima differed significantly among species ($P < 0.0001$; Fig. 1).

Ants responded to increasing temperatures with a characteristic set of behaviors. As temperatures neared CT_{max}, ants became extremely
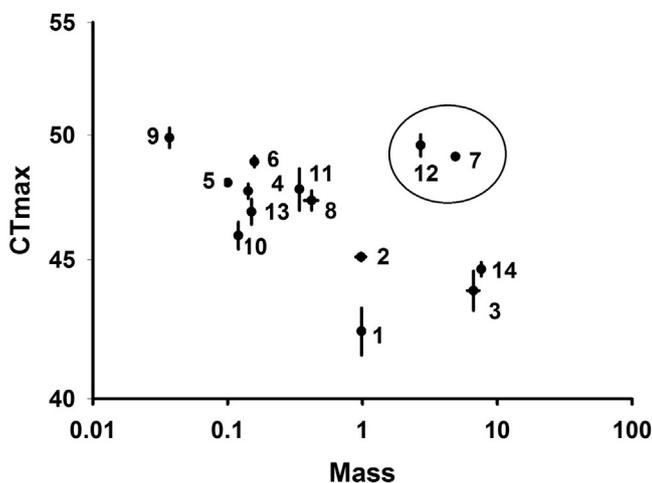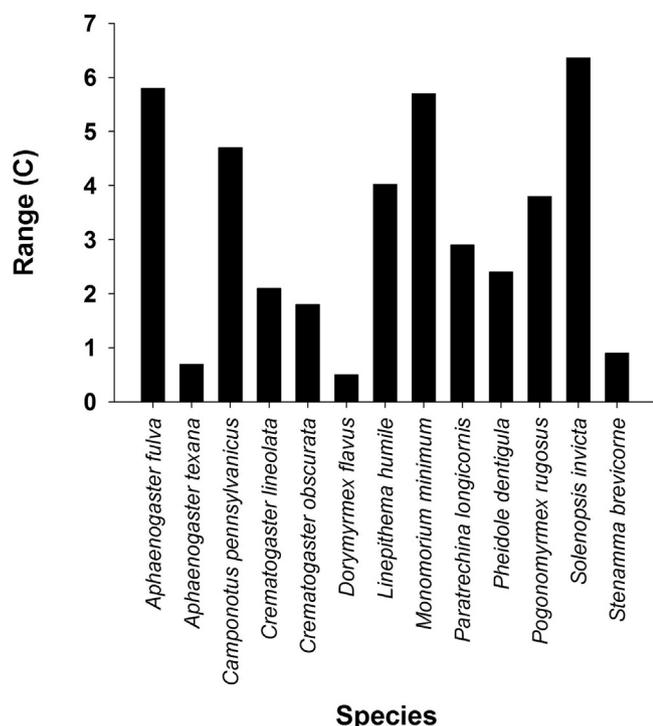
Fig. 2. Range of ant CT_{max} among colonies of the focal species. *Formica incerta* was omitted from this analysis since only a single colony was collected.

active and aggressive (e.g., biting one another; also see Friedlander et al., 1976). During the final minute prior to reaching CT_{max}, ant activity decreased conspicuously, and individuals became clumsy and had difficulty righting themselves. Upon removal from the water bath, ants regained mobility and stood upright within ten minutes. Individuals in the five *C. lineolata* colonies that were exposed to high temperatures a second time exhibited LORR at higher temperatures than during the previous trial (average difference among individuals = +1.8 °C, range = 0.8–2.7 °C; paired-t = 5.91, df = 4, P = 0.002).

4. Discussion

Here we demonstrate that thermal tolerance limits differ among common ant species of temperate zone forests and rangelands in Arkansas and Texas. We also show that differences in critical thermal maxima among colonies within a species are relatively small. In addition, differences in thermal maxima are of great importance in the face of changing climates, as the likelihood and duration of hot summer temperatures have increased in North America (Hansen et al., 2012); insects that can survive at higher temperatures may possess a significant competitive advantage in these new environmental conditions. In addition, insects that have lower thermal maxima and organisms that rely on these insects may face decreases in activity windows related to warmer environmental conditions.

In contrast to the interspecific and inter-colony results, the data did not support our expectation of a positive relationship between body size and CT_{max} among species; indeed, we found the opposite pattern. In ectotherms, larger body size generally buffers individuals from the effects of high temperatures (Beament, 1959). Body size often is ignored in studies of critical thermal maxima (Chown et al., 2002), and few data exist regarding the relationship between CT_{max} and body size in insects. Studies specifically examining ant body size in this context found either positive correlations between mass and CT_{max} (Cerda et al., 1998; Kaspari et al., 2014) or mixed relationships by subfamily (Oberg et al.,

2012). Given these unclear relationships, mass or mass surrogates should be included in analyses of thermal physiology, particularly in taxa such as ants, where body size can span three orders of magnitude (e.g., Kaspari and Weiser, 1999). Ant body size tends to increase with latitude at least regionally (Cushman et al., 1993; Blackburn et al., 1999), so size may be a confounding factor in regional or continental comparisons (e.g., Diamond et al., 2012). The short internal branches and extremely long terminal branches of the pruned phylogeny for species included in this study resulted in an overall lack of phylogentic signal in these physiological data. Thus, for these analyses conventional least squares regression and PGLS yield the same result. However, it is important to account for phylogenetic relationships among species when examining evolutionary patterns of phenotypic variation among species.

Interestingly, our results show that smaller ants have higher thermal tolerances than larger ants (Fig. 1), which contrasts with similar studies of both tropical ants (Kaspari et al., 2014) and temperate ants (Oberg et al., 2012). The reasons for this difference require further study, but we speculate that smaller ants benefit from foraging at a broader range of temperatures than large ants (Lessard et al., 2009). The smaller ants examined in this study (e.g., *Monomorium minimum*) commonly occur in more open habitats and are generally more active during the hottest parts of the day than the larger ants (e.g., *Camponotus pennsylvanicus*) that were examined, suggesting that interspecific differences in behavior and general patterns of activity confounded our body size results. Other studies have noted similar differences in thermal properties of open habitat versus forest insects (Kimura, 2004).

Our intraspecific results are similar to patterns observed in other studies exploring thermal parameters within species (e.g., *Pogonomyrmex rugosus*, Lighton and Turner, 2004; *Camponotus pennsylvanicus, Crematogaster lineolata*, Oberg et al., 2012); however, we report different specific critical thermal maxima. Our CT_{max} values likely differ from theirs due to different ramping temperatures (Overgaard et al., 2012), collection sites (Angilleta et al., 2007), and acclimation temperatures (Chown et al., 2009). Thus, standardization of methods among studies clearly is an essential prerequisite for ensuring the validity of comparisons, as noted elsewhere (Lutterschmidt and Hutchison, 1997).

Narrow CT_{max} ranges within and among ant colonies suggest that heat tolerance is genetically determined in ants, as observed in other insects (Sorensen et al., 2001). This study is one of few to examine intra-colony variations in ant CT_{max} (but see Cokendolpher and Phillips, 1990; Clemencet et al., 2010). Studies of ant thermal physiology often do not account for both sources of error (i.e., within and among colonies), which could underestimate local and regional intraspecific variability in CT_{max}.

Results of the duplicate heating trials of *C. lineolata* workers suggest that short-term heat hardening can occur in this species. Although more data are needed to fully support this conclusion, the clear pattern in these preliminary tests was unexpected and suggests that further studies of heat hardening in ants would be a useful extension of this project. Additionally, future studies should examine the relationships between phylogeny, body size, and thermal limits in a wider suite of species. These results will contribute to a greater understanding of the effects of a warming climate on terrestrial arthropods.

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