

# Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae)

Kaitlin M. Baudier\*, Abigail E. Mudd, Shayna C. Erickson and Sean O'Donnell

Department of Biodiversity Earth and Environmental Science, Drexel University, 3245 Chestnut Street, PISB Building, Philadelphia, PA 19104, USA

## Summary

1. Models that predict organismal and population responses to climate change may be improved by considering ecological factors that affect species thermal tolerance. Species differences in microhabitat use can expose animals to diverse thermal selective environments at a given site and may cause sympatric species to evolve different thermal tolerances.

2. We tested the hypothesis that species differences in body size and microhabitat use (above- vs. below-ground activity) would correspond to differences in thermal tolerance (maximum critical temperatures:  $CT_{max}$ ). Thermal buffering effects of soil can reduce exposure to extreme high temperatures for below-ground active species. We predicted larger-bodied individuals and species would have higher  $CT_{max}$  and that species mean  $CT_{max}$  would covary positively with degree of above-ground activity. We used Neotropical army ants (Formicidae: Ecitoninae) as models. Army ants vary in microhabitat use from largely subterranean to largely above-ground active species and are highly size polymorphic.

3. We collected data on above- and below-ground temperatures in habitats used by army ants to test for microhabitat temperature differences, and we conducted  $CT_{max}$  assays for army ant species with varying degrees of surface activity and with different body sizes within and between species. We then tested whether microhabitat use was associated with species differences in  $CT_{max}$  and whether microhabitat was a better predictor of  $CT_{max}$  than body size for species that overlapped in size.

4. Microhabitat use was a highly significant predictor of species' upper thermal tolerance limits, both for raw data and after accounting for the effects of phylogeny. Below-ground species were more thermally sensitive, with lower maximum critical temperatures ( $CT_{max}$ ). The smallest workers within each species were the least heat tolerant, but the magnitude of  $CT_{max}$  change with body size was greater in below-ground species. Species-typical microhabitat was a stronger predictor of  $CT_{max}$  than body size for species that overlapped in size. Compared to the soil surface, 10-cm subsoil was a significantly moderated thermal environment for below-ground army ants, while maximum surface raid temperatures sometimes exceeded  $CT_{max}$  for the most thermally sensitive army ant castes.

5. We conclude sympatric species differences in thermal physiology correspond to microhabitat use. These patterns should be accounted for in models of species and community responses to thermal variation and climate change.

**Key-words:** caste, eye size, microclimate, soil thermal buffering, thermal sensitivity

## Introduction

Predicting how animal populations and species respond to changing ambient temperatures is potentially complicated

by the array of thermal microhabitats used by animals at any given site. Examples of microhabitat-driven thermal clines include those from canopy to forest floor (Hood & Tschinkel 1990; Weiser *et al.* 2010; Kaspari *et al.* 2014), patches of shade vs. sun (Huey *et al.* 1989; Meisel 2004; McGlynn *et al.* 2010; Kaspari *et al.* 2014) and the effect

\*Correspondence author. E-mail: kmb478@drexel.edu

of soil thermal buffering relative to surface conditions (Zimmerman *et al.* 1994; Bulova 2002; Kumar & O'Donnell 2009). Current climate models rely largely on temperature data from weather stations placed 1–2 m above-ground in the open (Hijmans *et al.* 2005; Strauss, Formayer & Schmid 2013). Standard weather data collection is inadequate for assessing the diversity of thermal environments animals experience within sites (Porter *et al.* 1973; Kaspari 1993; Meisel 2004; McGlynn *et al.* 2010; Andrew *et al.* 2013). Microhabitat-specific thermal tolerance data may be critical for understanding how terrestrial animal populations are adapted to and limited by temperature, and how they will respond to directional change (Huey *et al.* 2012). We suggest that thermally distinct microhabitats can select for different thermal physiologies among sympatric species within a taxon. Our goal was to test whether species that use thermally distinct microhabitats differ in their upper thermal tolerance limits.

As small-bodied, ecologically dominant ectotherms, ants serve as useful models for understanding responses to temperature variation and climate change (Harkness & Wehner 1977; Hölldobler & Wilson 1990; Andrew *et al.* 2013). Here, we develop and test the hypothesis that microhabitat use can predict species differences in the upper limits of thermal tolerance (maximum critical temperature, henceforth  $CT_{max}$ ). We took advantage of the diversity of microhabitat use among Neotropical army ant species (Ecitoninae: Brady 2003) to test whether microhabitat use predicts species differences in  $CT_{max}$ . Sympatric army ant species (co-occurring at the same geographic site) range from largely above-ground activity to below-ground activity (Rettenmeyer 1963).

We used  $CT_{max}$  as a measure of upper thermal tolerance. An organism's thermal tolerance is the range of temperatures over which it maintains the capacity to function (Huey & Stevenson 1979; Angilletta, Niewiarowski & Navas 2002). Species and individuals vary in their thermal sensitivity, or the relative width of this thermal tolerance (Sunday, Bates & Dulvy 2011; Kaspari *et al.* 2014).  $CT_{max}$  is the maximum temperature at which an organism is able to survive, setting the upper bounds of animals' thermal tolerance (Huey & Stevenson 1979; Ribeiro, Camacho & Navas 2012).  $CT_{max}$  is often close to optimum performance temperatures, particularly among thermal specialists with narrow thermal tolerance ranges (Sunday, Bates & Dulvy 2011; Huey *et al.* 2012). In the absence of other physiological data,  $CT_{max}$  can serve as an informative criterion for predicting species' geographic ranges, and for predicting costs associated with local temperature increases that result from climate change (Sunday, Bates & Dulvy 2011; Huey *et al.* 2012; Kaspari *et al.* 2014).

We used species of army ants (subfamily Ecitoninae), a monophyletic lineage, as subjects (Brady 2003). Army ant species comparisons control for genetic variation to some extent. We used the well-supported molecular-based ecitonine phylogeny to account for potential species relatedness effects on thermal physiology (Felsenstein 1985;

Brady 2003; Brady *et al.* 2014). Neotropical army ant species share a combination of social, physiological and ecological attributes that make them tractable models for addressing microclimatic questions. All Ecitoninae are top predators that conduct colony activities (foraging, colony migrations, temporary nest formation) in massive coordinated social groups (Wheeler 1921; Schneirla 1933; Schneirla, Brown & Brown 1954; Rettenmeyer 1963). Importantly, species of Ecitoninae vary in degree of subterranean activity (Rettenmeyer 1963; Kumar & O'Donnell 2009), enabling us to assess how variation in surface vs. subterranean activity corresponded to thermal tolerance. The nine army ant species assayed in this study represent a range from among the most subterranean to the most surface-active Ecitoninae. We expected above-ground species would more often encounter extreme high temperatures that are potentially limiting to worker performance or survival, while the thermal buffering effects of subterranean microhabitats would ameliorate selection for high-temperature tolerance. Therefore, we predicted the most above-ground species would have the greatest heat tolerances, with lower heat tolerance in ants that spend more time underground.

All army ant species have size-polymorphic workers, but some species overlap widely in body size distributions (Rettenmeyer 1963; O'Donnell *et al.* 2011). This enabled within- and between-species analyses of body size effects on thermal tolerance (Clemencet *et al.* 2010; Oberg, Toro & Pelini 2012; Ribeiro, Camacho & Navas 2012; Kaspari *et al.* 2013). Within-species body size variation is related to thermal tolerance in many ant species (Clemencet *et al.* 2010; Ribeiro, Camacho & Navas 2012): upper thermal tolerance limits ( $CT_{max}$ ) generally increases with body size in ants, but the relationship between body size and  $CT_{max}$  varies substantially among ant taxa (Oberg, Toro & Pelini 2012). We asked whether microhabitat use was a better predictor of  $CT_{max}$  than body size for army ant species that overlapped in worker size distributions.

## Materials and methods

### ARMY ANT SUBJECTS

We collected and assayed army ants during the rainy season (June–August) of 2013 and 2014 in and around Monteverde, Costa Rica (N10° 17' 55", W84° 48' 60"). We conducted maximum thermal tolerance ( $CT_{max}$ ) assays using 972 army ant workers collected from 47 colonies of nine species in three genera (Table 1). All species were previously described with the exception of one which keys to *Labidus praedator* (Smith) but is morphologically distinct in having a shining cuticle. These ants are strongly differentiated genetically from *L. praedator* (unpublished CO1 sequence data) and are broadly sympatric with *L. praedator*; we treated them as a separate species, *Labidus* JTL001 (J.T. Longino, pers. comm. 2014).

Subjects were collected at elevations from 1283 m above sea level (m a.s.l.) to 1711 m a.s.l. in montane forests. At each army ant sample site, we recorded latitude/longitude coordinates and elevation using hand-held global positioning system (GPS) units. The GPS

**Table 1.** Individual and colony sample sizes for  $CT_{max}$  assays for nine species in the genera *Eciton*, *Labidus* and *Neivamyrmex*; average and standard deviation of  $CT_{max}$  for each species (used for phylogenetic correction); percentage of ants in each species found to be below the maximum raid temperature measured; smallest caste ratio of eye to head width at antennal insertion (RESI) used as a continuous proxy for soil habitat use; and species habitat use based on bivouacking and raiding above- vs. below-ground

Species	Worker <i>N</i>	Colony <i>N</i>	Mean $CT_{max}$	$CT_{max}$ SD	% $CT_{max}$ <36 °C	RESI	Microhabitat use
<i>E. b. parvispinum</i>	359	17	40.2	1.5	1.1	0.12	Above <sup>a,b,c</sup>
<i>E. mexicanum</i>	60	3	38.2	1.3	1.7	0.14	Intermediate <sup>b</sup>
<i>L. coecus</i>	135	7	35.6	2.5	52.6	0.05	Below <sup>b,d,e</sup>
<i>L. praedator</i>	59	3	36.4	2.4	6.5	0.08	Intermediate <sup>b,f</sup>
<i>L. JTL001</i>	77	3	37.5	1.5	32.2	0.07	Intermediate <sup>g,h</sup>
<i>L. spininodis</i>	19	1	35.4	1.5	57.9	0.05	Intermediate <sup>h</sup>
<i>N. macrodentatus</i>	19	1	32.4	2.9	79.0	0	Below <sup>h,i,j</sup>
<i>N. pilosus</i>	24	1	39.0	0.9	0	0.10	Intermediate <sup>b</sup>
<i>N. sumichrasti</i>	220	11	38.2	1.4	6.4	0.08	Intermediate <sup>k</sup>
Grand total	972	47					

<sup>a</sup>Schneirla, Brown & Brown (1954), <sup>b</sup>Rettenmeyer (1963), <sup>c</sup>O'Donnell *et al.* (2011), <sup>d</sup>Kumar & O'Donnell (2009), <sup>e</sup>Perfecto (1992), <sup>f</sup>O'Donnell *et al.* (2007), <sup>g</sup>Longino (pers. comm., 2014), <sup>h</sup>Pers. Obs., <sup>i</sup>Menozi (1931), <sup>j</sup>Watkins (1968) and <sup>k</sup>Dunn (2003).

unit elevations were averaged with those generated from collected GPS coordinates by using a digital elevation model data base (<http://www.gpsvisualizer.com/elevation>). We located surface raids by walking cleared trails in search of ant columns (O'Donnell *et al.* 2007, 2011; Kumar & O'Donnell 2009). When a column was encountered, we followed it in the opposite direction of portered food to reach the raid front. Whenever possible, we aspirated workers from raid fronts or raiding columns of ants near the front; some workers were also collected near bivouacs.

Underground-raiding army ants were sampled via standard sunken traps baited with tuna oil (O'Donnell & Kumar 2006; O'Donnell *et al.* 2007; Kumar & O'Donnell 2009). A bulb planter was used to excavate a cylindrical hole *c.* 10 cm in depth and 10 cm diameter. Tuna oil was applied to the bare soil on the bottom of the hole so as to allow underground foragers to enter via tunnels from any side, including below. The hole was then covered with a 200-mL plastic cup and a plastic plate to create a darkened environment and to provide some protection from surface-foraging species. At each site, 5–10 baits spaced five metres apart were placed in a transect alongside permanent trails, 1–5 m from the trail. Baits were checked every two to four hours until army ants were observed raiding in the trap pits.

#### TEMPERATURE MEASUREMENTS

We sampled soil temperatures at soil surface and 10 cm below the surface (the depth at which the ants were baited and collected). Observations of subterranean foraging depths for most army ant species are anecdotal and vary from several meters deep to beneath constructed soil tubes on the surface (Rettenmeyer 1963; Perfecto 1992). Foraging depths near the surface likely experience the most extreme fluctuations in dial temperature and humidity (Parton & Logan 1981). One to three pairs of thermal data loggers (ibutton; Maxim Integrated™, San Jose, CA, USA) were deployed along each bait transect at soil surface and 10 cm below the surface. Loggers recorded temperature every five minutes preceding, during and 3 days after *n* = 8 subsurface raid collections (three raids in 2013, five raids in 2014). We used temperatures collected every five minutes across 24 h of each day within each site to calculate daily mean, maximum, minimum and range in temperature.

Surface raid temperatures at above-ground raids were collected using hand-held infrared thermometers in 2014 (BAFX Products®, Milwaukee, WI, USA). All surface-accessible parts of 40 raids of five species were measured every 25 cm along the length of columns and across the width of raid fronts. All ibuttons were calibrated to within ±0.5 °C (the manufacturer-reported instrument error). Ibutton accuracy was confirmed with a thermocouple, and also by water bath (0 °C & 42 °C as measured by a certified mercury glass thermometer). Infrared thermometer readings of soil temperatures were within ±0.5 °C of temperatures recorded by ibuttons under field conditions.

#### BODY SIZE CATEGORIES

We divided workers from each species into categories based on visually apparent body size and caste differences. In the case of *Eciton burchellii parvispinum* (Forel), the categories were as follows: soldiers, porters, large workers and small workers (Franks 1985). For *Labidus spininodis* (Emery), *Neivamyrmex macrodentatus* (Menozi) and *Neivamyrmex pilosus* (Smith), workers were placed into two categories: large and small. We divided all other species: *Eciton mexicanum* (Roger), *Labidus coecus* (Latreille), *L. praedator*, *L. JTL001*, and *Neivamyrmex sumichrasti* (Norton), into small, medium and large worker categories. After  $CT_{max}$  assays were complete, workers were stored together in 95% ethanol for later morphological measurements; workers from each size category per species were pooled. We photographed worker head capsules within each size category lying flat along the frontal plane using a digital camera (magnifications from 10× to 60×, depending on head size) to generate 1200 × 900 pixel images. We then used IMAGE J software (version 1.45s; <http://imagej.nih.gov/ij/>) to measure head width at antennal insertions. We used the mean head capsule width of workers from each size category as an index of body size (Franks 1985; Weiser & Kaspari 2006). Within each species, we conducted either a Student's *t*-test (species with two size categories) or an ANOVA with post hoc Tukey test (species with three or more size categories), using linear models in R, to confirm that the visually discerned body size categories differed significantly in head width. We used mean head capsule width for each worker category as the predictor variable in the analysis of  $CT_{max}$  differences among size categories.

## THERMAL TOLERANCE ASSAYS

We used standard dynamic methods to measure each ant's critical thermal maximum ( $CT_{max}$ ) (Lutterschmidt & Hutchison 1997; Diamond *et al.* 2012; Oberg, Toro & Pelini 2012). All assays were performed in a laboratory with ambient temperature similar to surrounding forest (in-laboratory temperatures recorded with ibuttons over two days: mean  $21.05 \pm 2.50$  °C SD, maximum 26 °C, minimum 17.5 °C). Time elapsed from collection to assay was recorded for each colony. Assays were conducted within 2 h of subjects being exposed to laboratory conditions, a standard practice that minimizes physiological acclimation to laboratory conditions (Oberg, Toro & Pelini 2012; Ribeiro, Camacho & Navas 2012), and within 5 h of subject collection from each raid site (mean  $1.756 \pm 0.878$  SD), to reduce effects of transit time. While in transit from the field, we supplied ants with water in excess (moistened paper towel) to prevent desiccation prior to performing the assays.

Subject ants were individually placed into 1.5-mL plastic Eppendorf tubes stoppered with a cotton ball and then secured with a cap to prevent ants from seeking thermal refuge during the assay (Oberg, Toro & Pelini 2012). Approximately 20 vials with ants from each colony were then inserted simultaneously into a digitally controlled aluminium heat block which had been pre-heated to 30 °C. One ant from each caste in every colony was held in a similar vial adjacent to the heat block at laboratory ambient temperature as a control. We increased heat block temperature 1 °C every 10 min, checking for movement at the end of each 10-min interval. The highest temperature at which an individual ant displayed movement in response to light tapping was considered its  $CT_{max}$ . Immobility is a standard conservative measure of  $CT_{max}$  for ants (Lutterschmidt & Hutchison 1997; Meisel 2004; Oberg, Toro & Pelini 2012).

## STATISTICAL ANALYSES

Unless otherwise stated, all analyses were performed in R version 2.15.2 (R Development Core Team 2011).

Predictor variables of  $CT_{max}$ 

We defined three categories of microhabitat use by new world army ants: 'above-ground' for species that bivouac and raid primarily above-ground, 'below-ground' for those that typically raid

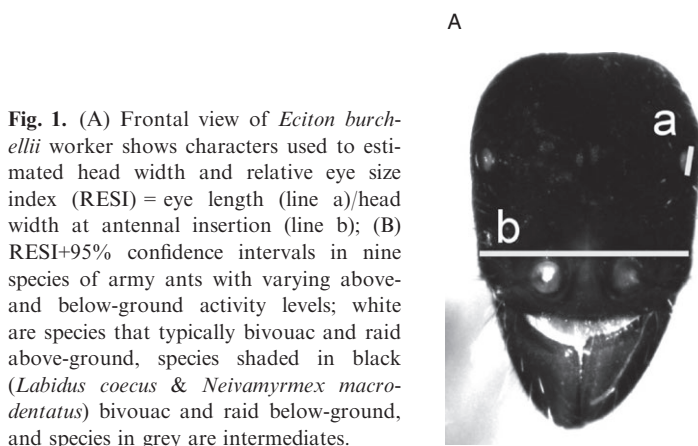
and bivouac below-ground and 'intermediate' for those that bivouac below-ground and raid either partly or entirely above-ground. However, the behaviours and activity budgets of some species are relatively poorly known, making precise categorization of microhabitat use difficult. As a proxy for microhabitat use, we developed a continuously varying morphological index of relative degree of above-ground activity for each species, the relative eye size index (RESI) (Fig. 1):

$$RESI = \text{external eye facet length/head width at antennal insertions}$$

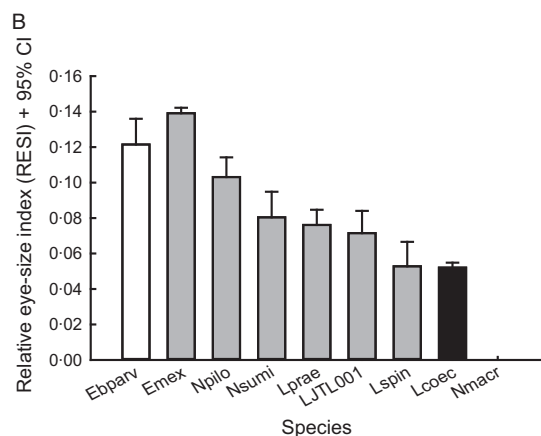
Eye size is an ecologically relevant morphological character that is predictive of environmental light exposure among many New World ant species (Weiser & Kaspari 2006). The army ant eye consists of a single highly reduced facet (ommatidium), thought to mostly function to sense light levels (Rettenmeyer 1963). Increased ability to sense light may be more adaptive for surface-foraging army ants, which actively avoid forest clearings and sun flecks (Levings 1983; Meisel 2006). To control for variation in relative eye size among worker size-castes, we calculated the mean RESI value from the five smallest ants we collected in each species. Vestigial eye spots with no external lens were given an eye length of 0. RESI corresponded closely to the microhabitat categories: more above-ground species generally had higher RESI values, with the most below-ground species having a RESI value of 0 (Fig. 1). To check the validity of RESI as an indicator of habitat use, we ran all analyses (except the phylogenetic correction analysis) using habitat-use category in place of RESI and obtained similar results in all cases; we do not present the results of these analyses.

Testing predictors of  $CT_{max}$ 

We used multifactor, mixed-model analyses of variance to identify significant predictors of  $CT_{max}$  (Quinn & Keough 2002). The full linear mixed-effects model (LME in R) included the potentially confounding covariates: t-elapsed (time elapsed from collecting to assay) and elevation (metres above sea level) as fixed factors. RESI (microhabitat use index) and head width were also included as fixed factors. Colony was treated as a random nested variable within species. Effects of species and colony on head width distribution were included as random predictors of  $CT_{max}$ . We used restricted maximum likelihood and maximum likelihood estimations (via the ANOVA function) to identify and eliminate



**Fig. 1.** (A) Frontal view of *Eciton burchellii* worker shows characters used to estimate head width and relative eye size index ( $RESI = \text{eye length (line a)/head width at antennal insertion (line b)}$ ); (B)  $RESI + 95\%$  confidence intervals in nine species of army ants with varying above- and below-ground activity levels; white are species that typically bivouac and raid above-ground, species shaded in black (*Labidus coecus* & *Neivamyrmex macrodentatus*) bivouac and raid below-ground, and species in grey are intermediates.



non-significant random and fixed variables, respectively. Sampling year effects on  $CT_{\max}$  were tested by comparing the previously reduced model with and without year as a fixed variable for the subset of species sampled in both years. If a potentially confounding covariate had a significant effect, we included it in the reduced model; significance tests for the effects of predictor variables of interest account for these covariates.

### Phylogenetic correction

The use of RESI as a continuously varying proxy for microhabitat allowed us to perform phylogenetic corrections. We used COMPARE software version 4.6b (Martins 2004) to analyse  $CT_{\max}$  relationships with microhabitat with phylogenetic generalized least squares (PGLS) regressions based on Felsenstein's independent contrasts approach (Felsenstein 1985; Martins & Hansen 1997). COMPARE software version 4.6b was used (Martins 2004). We based the PGLS regression on the genus-level phylogeny of Ecitoninae (Brady 2003; Brady *et al.* 2014) (Fig. S1, Supporting Information). All branch lengths were set to one except branch lengths within species-level polytomies, which were set to a small value (0.001) as recommended by Martins (2004); this step was necessary because PGLS analysis requires a fully resolved phylogeny.

### Temperature data

We compared daily maximum, minimum and temperature range differences between soil surface and 10-cm below-ground using a mixed-effects model (Bates *et al.* 2014), with transect as a random variable and above- vs. below-ground as a fixed-factor predictor variable.

## Results

### VALIDATING THE METHODS

Head width differed significantly among our body size categories (ANOVA,  $P < 0.0001$ ,  $F_{24, 982} = 200.36$ ; this analysis included  $CT_{\max}$  subjects and control ants). All size categories within each species differed significantly in head width (ANOVA + post hoc Tukey or Student's *t*-tests; all pairwise comparisons  $P < 0.05$ ). However, there was considerable overlap in head size between species. About 220 of 235 control ants (94%) survived in the laboratory throughout the duration of the assays, indicating holding ants in the assay tubes at ambient temperatures caused little mortality during the time required to assess  $CT_{\max}$ .

### ENVIRONMENTAL TEMPERATURES

Surface and 10-cm subsoil probes differed significantly in all air temperature measures: average daily temperature ( $\chi^2 = 224.7$ , d.f. = 1,  $P < 0.001$ ), maximum daily temperature ( $\chi^2 = 13.7$ , d.f. = 1,  $P < 0.001$ ), minimum daily temperature ( $\chi^2 = 78.6$ , d.f. = 1,  $P < 0.001$ ) and daily temperature range ( $\chi^2 = 56.1$ , d.f. = 1,  $P < 0.001$ ) (Table 2, Fig. 2). Among all transects and days, air temperatures ranged at soil surface from 16 to 23 °C, with a range of 17.5–20 °C at depths of 10 cm.

High temperatures recorded at 40 above-ground raids with IR thermometers were 36.1 °C for *N. sumichrasti*, 30 °C for *E. b. parvispinum*, 28.8 for *L. praedator*, 20.1 °C for *L. JTL001* and 18.3 for *E. mexicanum* (Table S1, Supporting Information). The most extreme surface temperature of 36.1 °C, measured at an *N. sumichrasti* raid, was greater than  $CT_{\max}$  values measured for 11.83% of that species and exceeded  $CT_{\max}$  for some individual workers in other species as well (Fig. 3; Table 1; Table S1).

### PREDICTORS OF $CT_{\max}$

$CT_{\max}$  values did not differ significantly between sample years among the four species sampled in both years ( $\chi^2 = 3.544$ , d.f. = 1,  $P = 0.060$ ). Time elapsed between collection and assay was a significant predictor of  $CT_{\max}$  ( $\chi^2 = 6.12$ , d.f. = 1,  $P = 0.013$ ), so time to assay was corrected for in all statistical models. Both microhabitat use (as indexed by RESI) and body size were significant positive predictors of  $CT_{\max}$  (Figs 4 and 5).  $CT_{\max}$  was positively associated with colony differences in head width ( $\chi^2 = 141.6$ , d.f. = 3,  $P < 0.001$ ) and species differences in head width ( $\chi^2 = 24.5$ , d.f. = 3,  $P < 0.001$ ). Elevation was not a significant predictor of  $CT_{\max}$  ( $\chi^2 = 3.1$ , d.f. = 1,  $P = 0.080$ ). The interaction between microhabitat and head width was a significant predictor of  $CT_{\max}$  ( $\chi^2 = 4.047$ , d.f. = 1,  $P = 0.044$ ), indicating that smaller subterranean ants were most sensitive to high temperatures (Fig. 5).

### PHYLOGENETIC CORRECTION

$CT_{\max}$  was positively associated with degree of above-ground activity after phylogenetic correction. RESI covaried positively with species mean  $CT_{\max}$  (raw data

**Table 2.** Average daily maximum, minimum, mean and range in temperature (°C ± SD) as recorded by ibutton thermal probes placed at soil surface and 10-cm subsurface, across 8 bait transects sampled in 2013 and 2014

Soil depth	Max	Min	Mean	Range	<i>N</i> Days	<i>N</i> Points
0 cm	19.49 ± 1.08	17.38 ± 0.62	18.23 ± 0.44	2.11 ± 1.24	38	14 216
10 cm	18.87 ± 0.32	18.61 ± 0.31	18.71 ± 0.28	0.26 ± 0.28	38	14 217
Grand total						28 433

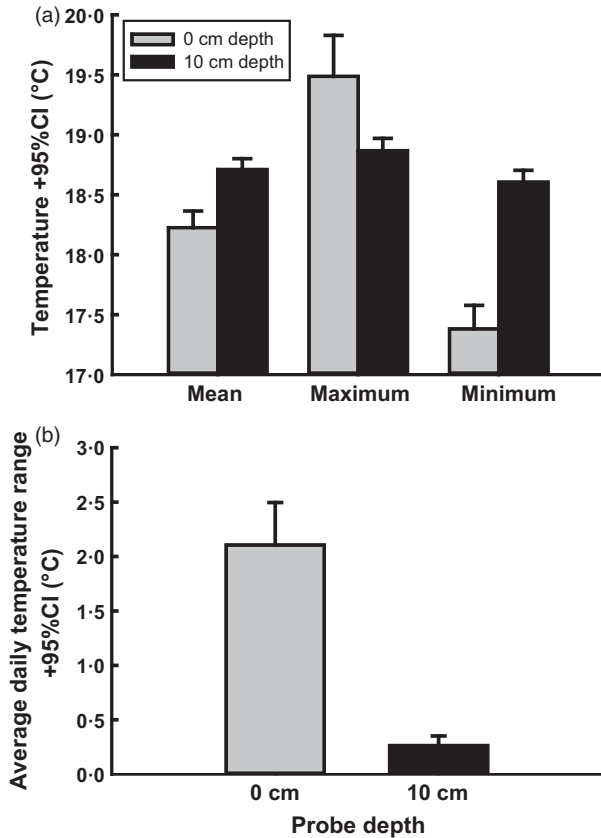


Fig. 2. (a) Average daily mean temperatures, daily maximum temperatures and daily minimum temperatures with 95% confidence intervals, collected from temperature probes at two soil depths; (b) Difference in average daily temperature range among two different soil depths.

$R = 0.89$ ,  $N = 9$ ,  $P = 0.001$ ; PGLS regression:  $R = 0.88$ ,  $N = 8$ ,  $P = 0.004$ ; Fig. 4).

## Discussion

### MICROHABITAT USE AND THERMAL TOLERANCE

Species-typical microhabitat use (above- vs. below-ground activity) was highly significantly associated with species differences in heat tolerance (Figs 4 and 5). In general,  $CT_{max}$  was higher in the most above-ground species (*E. b. parvispinum*), lower in the most below-ground species (*Labidus coecus* and *N. macrodentatus*) and intermediate in the other sampled species which are active both underground and on the surface (Rettenmeyer 1963) (Table 1). This suggests greater heat tolerance is more adaptive for above-ground species due to higher maximum temperatures experienced in the surface environment.

### WITHIN- AND BETWEEN-SPECIES BODY SIZE EFFECTS

Thermal tolerance generally increases with body size in ants (Cerdá & Retana 2000; Clemencet *et al.* 2010;

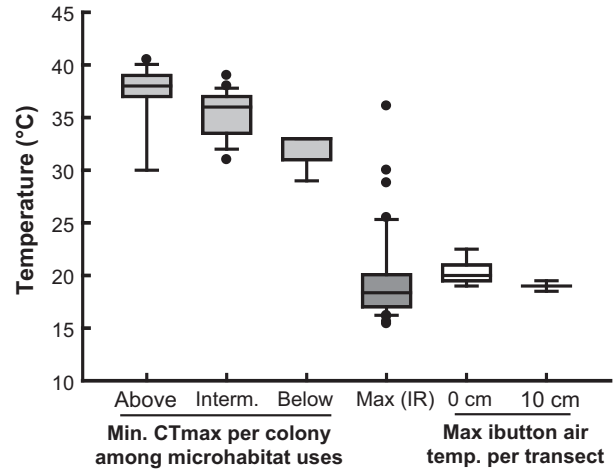


Fig. 3. Differences between  $CT_{max}$  values and two types of environmental temperature measurements: ibutton data-logger measurements and maximum infrared (IR) surface raid temperatures; light grey are minimum colony  $CT_{max}$  values for each habitat group, dark grey are maximum surface raid temperatures recorded, and white are ibutton bait trap temperatures recorded at soil surface and 10-cm depth.

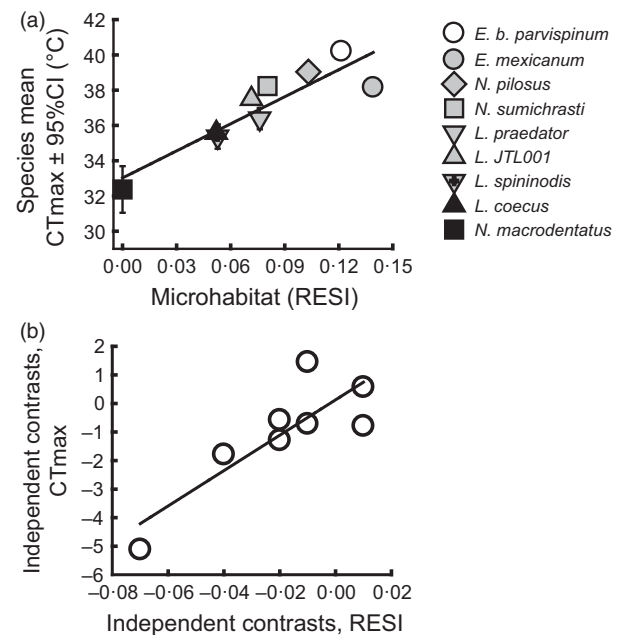


Fig. 4. (a) Phylogenetically uncorrected correlation between average maximum critical temperature ( $CT_{max}$ ) and relative eye size index (RESI) among nine species of army ants; white symbols indicate the species typically raid and bivouac above-ground, black indicates raiding and bivouacking typically below-ground, and grey indicates species that bivouac below-ground but at least commonly raid above-ground; circles are species of *Eciton*, triangles are species of *Labidus*, and squares are species of *Neivamyrmex*; (b) Independent contrasts showing significant (and similar) relationship between RESI and  $CT_{max}$  after accounting for relatedness.

Ribeiro, Camacho & Navas 2012). We showed that small workers were less heat tolerant in highly polymorphic army ants (Fig. 5). Several physiological factors related to

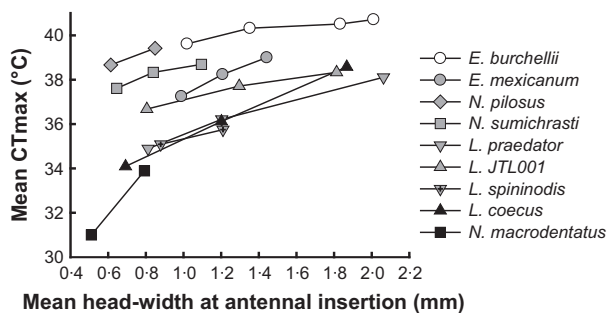


Fig. 5. Relationship between  $CT_{max}$  and body size among nine species of army ants with three different uses of soil microhabitat; white symbols indicate the species typically raid and bivouac above-ground, black indicates raiding and bivouacking typically below-ground, and grey indicates species that bivouac below-ground but at least commonly raid above-ground; circles are species of *Eciton*, triangles are species of *Labidus*, and squares are species of *Neivamyrmex*.

body size may affect size-caste differences in  $CT_{max}$ . Variation in heat-shock protein synthesis affected  $CT_{max}$  differences between highly thermophilic *Cataglyphis* species (Gehring & Wehner 1995). Differences in cuticular thickness may also be relevant, as reduced cuticle thickness is known to cause increased thermal conductance among insects (Galushko *et al.* 2005). The interplay between body size (surface area to volume ratio) and cuticular lipid content affects thermal tolerance in ants adapted to desiccation-prone microhabitats (Hood & Tschinkel 1990).

$CT_{max}$  increased with species mean body size in several ant subfamilies (Clemencet *et al.* 2010; Oberg, Toro & Pelinesi 2012; Ribeiro, Camacho & Navas 2012). Our data suggest small army ant species were less able to survive extreme high temperatures than larger species. However, there was considerable overlap in body size categories between some army ant species we sampled, enabling comparisons of workers with similar body sizes across species. The effects of body size on maximum thermal tolerance were more extreme in more below-ground species. For example, small workers of the most subterranean species (*L. coecus* and *N. macrodentatus*) were less thermally tolerant than expected from their body size relative to the other army ants we studied (Fig. 5). This difference in the relationship between body size and  $CT_{max}$  among species indicates that high thermal tolerance in small workers is more adaptive in surface-dwelling species. Small ant workers reach equilibrium with ambient temperatures faster than larger nest mates; coupled with greater thermal sensitivity (lower  $CT_{max}$ ), this suggests brief exposure to high temperatures is especially costly to small workers (Kaspari *et al.* 2014).

#### ENVIRONMENTAL TEMPERATURES

We predicted that even a shallow soil depth would buffer thermal variation relative to the ground surface and that

this selective pressure has resulted in higher  $CT_{max}$  among more epigeic species. We found that 10 cm of soil caused significant thermal buffering at baits where below-ground raiding species were collected (Table 2, Fig. 2). Although anecdotal reports suggest subterranean army ants such as *L. coecus* can forage much deeper (Longino pers. comm. 2014), even at this modest depth, workers experienced a distinct thermal environment that was moderated in temperature. In our ibutton sampling, below-ground daily average temperatures were slightly warmer than surface temperatures; however, maximum surface temperatures were significantly higher than those recorded underground, suggesting that occasional spikes in ambient temperatures above-ground may be a selective force on army ant  $CT_{max}$ .

There was high temporal and spatial variability in surface temperatures experienced by above-ground and intermediate army ant species (those with medium to high RESI values). Maximum ground-surface temperatures recorded at a high-elevation raid of *N. sumichrasti* exceeded  $CT_{max}$  values for many small intermediate habitat-use army ants and some small workers of above-ground foraging species and exceeded  $CT_{max}$  of most individuals in below-ground species (Fig. 3; Table 1). Although below-ground species are less likely to interact with these extreme temperatures, future increased incidence and duration of high surface temperatures are potentially limiting to species with intermediate and surface habitat usage patterns.

It is important to note that the criterion we used for exceeding  $CT_{max}$  (total immobility) is a conservative measure (Lutterschmidt & Hutchison 1997). Worker physiological impairment and colony fitness costs therefore may occur at temperatures below a species'  $CT_{max}$  as reported by this study. Deviations from optimum temperatures for tropical species with narrow thermal tolerances can impose high fitness costs even without causing observable mortality (Kingsolver, Diamond & Buckley 2013).

#### ECOLOGICAL IMPLICATIONS

We demonstrated that temperature differences caused by soil microclimates can select for species diversity in heat tolerance among montane Ecitoninae. Population responses to directional climate change include shifts in latitudinal and elevational range (Colwell *et al.* 2008; Deutsch *et al.* 2008; Longino & Colwell 2011). However, patterns of range shifts can differ among species (Deutsch *et al.* 2008; Corlett 2011). Some of these species differences may be due to species-typical microhabitat use. We expect that future exploration of how populations differ across elevational gradients will show higher  $CT_{max}$  in lowland Ecitoninae. This work is relevant to predicting potential for upward elevational shifts of army ant populations. We predict sympatric army ants (and other ectotherms) that occupy thermally distinct microclimates will experience different pressures as climate warms.

Although climate change models predict less extreme temperature changes at low latitudes, the relatively narrow

thermal tolerances among tropical species could exacerbate climate change impacts in the tropics (Deutsch *et al.* 2008; Sunday, Bates & Dulvy 2011; Chown 2012; Huey *et al.* 2012). We demonstrated that even at the highest elevations reported for surface-raiding army ants (over 1700 m a.s.l.), intermediate surface-raiding ants can encounter temperatures that challenge the thermal physiologies of their most sensitive castes. This indicates that a small directional change in climate may have a large impact on these species that already operate near the maximum of their thermal tolerance ranges. Army ants are abundant top predators that can strongly impact density, diversity and patchiness of their prey animals (Kaspari & O'Donnell 2003; Kaspari *et al.* 2011), and army ant colonies host diverse symbionts (Rettenmeyer 1962; Rettenmeyer *et al.* 2011). Our data suggest the effects of directional thermal change will vary among army ant species, potentially causing complicated alterations of forest community structure via effects on their prey and on their symbionts.

$CT_{max}$  is a commonly used measure of thermal sensitivity (Rezende, Tejedo & Santos 2011; Ribeiro, Camacho & Navas 2012). However,  $CT_{max}$  varied little along elevational and latitudinal thermal gradients relative to variation in minimum critical temperatures ( $CT_{min}$ ) in diverse terrestrial ectotherms (Gaston & Chown 1999; Sunday, Bates & Dulvy 2011; Hoffmann, Chown & Clusella-Trullas 2013; Rezende, Castañeda & Santos 2014). Our findings show that  $CT_{max}$  can vary significantly on much smaller spatial scales, even among closely related taxa. This suggests that stability in thermally buffered soil temperatures vs. the heterogeneity and variability of surface temperature may play a key role in the evolution of heat tolerance in ectotherms that occupy these distinct microclimates.

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## Data accessibility

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.7q3j8> (Baudier *et al.* 2015).

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Fig. S1.** Phylogeny used for comparative analysis; generic-level separation based on phylogenies (Brady 2003; Brady et al. 2014).

**Table S1.** Summary of maximum infrared temperature readings taken at raids of several above-ground foraging army ants.