

# Thermal adaptation generates a diversity of thermal limits in a rainforest ant community

MICHAEL KASPARI<sup>1</sup>, NATALIE A. CLAY<sup>1,2</sup>, JANE LUCAS<sup>3</sup>, STEPHEN P YANOVIK<sup>4</sup> and ADAM KAY<sup>3</sup>

<sup>1</sup>Graduate Program in Ecology and Evolution, Department of Biology, University of Oklahoma, Norman, OK 73019, USA,

<sup>2</sup>Smithsonian Tropical Research Institute, Balboa, Panama, <sup>3</sup>Department of Biology, University of St. Thomas, St. Paul, MN 55105, USA, <sup>4</sup>Department of Biology, University of Louisville, Louisville, KY 40292, USA

## Abstract

The Thermal Adaptation Hypothesis posits that the warmer, aseasonal tropics generates populations with higher and narrower thermal limits. It has largely been tested among populations across latitudes. However, considerable thermal heterogeneity exists within ecosystems: across 31 trees in a Panama rainforest, surfaces exposed to sun were 8 °C warmer and varied more in temperature than surfaces in the litter below. Tiny ectotherms are confined to surfaces and are variously submerged in these superheated boundary layer environments. We quantified the surface  $CT_{min}$  and  $CT_{max}$ s (surface temperatures at which individuals grew torpid and lost motor control, respectively) of 88 ant species from this forest; they ranged in average mass from 0.01 to 57 mg. Larger ants had broader thermal tolerances. Then, for 26 of these species we again tested body  $CT_{max}$ s using a thermal dry bath to eliminate boundary layer effects: body size correlations observed previously disappeared. In both experiments, consistent with Thermal Adaptation,  $CT_{max}$ s of canopy ants averaged 3.5–5 °C higher than populations that nested in the shade of the understory. We impaled thermocouples in taxidermy mounts to further quantify the factors shaping operative temperatures for four ant species representing the top third (1–30 mg) of the size distribution. Extrapolations suggest the smallest 2/3rds of species reach thermal equilibrium in <10s. Moreover, the large ants that walk above the convective superheated surface air also showed more net heating by solar radiation, with operative temperatures up to 4 °C higher than surrounding air. The thermal environments of this Panama rainforest generate a range of  $CT_{max}$  subsuming 74% of those previously recorded for ant populations worldwide. The Thermal Adaptation Hypothesis can be a powerful tool in predicting diversity of thermal limits within communities. Boundary layer temperatures are likely key to predicting the future of Earth's tiny terrestrial ectotherm populations.

**Keywords:** ants, boundary layer, canopy, community, ectotherms, thermal limits, tropical forest, understory

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## Introduction

Organism's are characterized by their thermal limits (the minimum,  $CT_{min}$ , and maximum  $CT_{max}$  temperature – typically body temperature – at which an organism maintains muscular control, Huey & Stevenson, 1979). A basic tenet of thermal ecology is that these limits, combined with a map of available microclimates, help predict an organism's vitality and distribution (Huey & Slatkin, 1976; Tracy & Christian, 1986; Root, 1988; Zimmermann *et al.*, 2009; Sunday *et al.*, 2012). Yet a general model for thermal limits – how and why  $CT_{min}$  and  $CT_{max}$  are distributed across taxa in time and space – remains elusive (Angilletta, 2009).

Toward this end, meta-analyses have matched thermal limits of a population to the average temperature of its locality. They have been instrumental in

developing the Thermal Adaptation Hypothesis: a collection of two working hypotheses key to climate change biology (Chown *et al.*, 2002; Deutsch *et al.*, 2008; Huey *et al.*, 2009; Sunday *et al.*, 2011; Diamond *et al.*, 2012b; Kellermann *et al.*, 2012). The first assumes a trade-off between adaptations that lower thermal limits and those that raise them (Hazel, 1995; Portner, 2001; Hochachka & Somero, 2002) generating a positive correlation between  $CT_{max}$  and  $CT_{min}$  (Angilletta, 2009). The second posits that the machinery of thermal limits is costly and that warm aseasonal tropics should favor populations with higher thermal maxima and narrower thermal tolerances (Janzen, 1967; Lynch & Gabriel, 1987; Deutsch *et al.*, 2008; Huey *et al.*, 2009; Sunday *et al.*, 2011). Combined, these hypotheses predict limited acclimatization and adaptation of thermal maxima to a warming world, with tropical species at the greatest risk of extirpation. At the same time, these reviews have highlighted three gaps in our understanding of thermal limits.

Correspondence: Michael Kaspari, tel. +405 590 2151, fax +405 325 6211, e-mail: mkaspari@ou.edu

### *Challenges to quantifying the thermal ecology of tiny ectotherms*

First, a reliance on climate models in global change biology (Stocker *et al.*, 2013) often implicitly assumes that the well-mixed air in weather stations is an adequate stand-in for an organism's operative temperature (Sunday *et al.*, 2014). But the joke among microclimate folks is that the only microclimate adequately described by a weather station is that of the spider living in the weather station. The operative temperature of any terrestrial ectotherm is determined not only by *convection* (the transfer of heat between body and air) but also by the further interplay of *conductance* (the direct transfer of energy between physical objects), *evaporation*, *metabolic heat* and *radiation* (both from the sun and from the surface on which the organism walks, Harrison *et al.*, 2012). Tiny ectotherms comprise most of Earth's individuals and species (May, 1978). For them, surface temperatures may critically shape operative temperature (Oke, 1978; Bakken, 1992; Potter *et al.*, 2013). Boundary layer microclimates near surfaces, for example, can superheat relative to the air above (Andrew *et al.*, 2013; Oke, 1978; Porter & Gates, 1969; Potter *et al.*, 2013; Stevenson, 1985; see also Woods, 2013). Moreover, solar radiation can further heat organisms directly and indirectly (through a combination of radiation, conductance, and convection, Harrison *et al.*, 2012). Thus, there is ample reason to suspect that small ectotherms in sunny habitats will experience thermal environments that are warmer, or at least different, from larger organisms or those in the shade nearby.

A second, related, knowledge gap is the way body size shapes thermal tolerance (Chown *et al.*, 2002). All taxa vary in size (Brown, 1995) with consequences for thermal ecology. As ectotherms decrease in size, the relative importance of weather station temperature, radiative heating, and thermal inertia decrease (Porter & Gates, 1969; Willmer & Unwin, 1981; Stevenson, 1985; Tracy, 1992; Angilletta, 2009) and the importance of convection inside the boundary layer increases. In this way, many diverse invertebrate taxa (e.g., Coleoptera, Hemiptera, Hymenoptera, Lepidoptera) may have their smallest species experiencing warmer temperatures, than the larger ones that tower above them.

Finally, the plants, soils, and topography of any ecosystem generate a variety of microclimates (Porter & Gates, 1969; Potter *et al.*, 2013; Sunday *et al.*, 2014). For example, lowland tropical rainforests include both the sunlit tropical canopy and the shaded forest floor 20–40 m below (Madigosky, 2004). Our experience has shown that a canopy branch exposed to the sun can be uncomfortable to the touch while its underside is cool. A diverse taxon with a range of body sizes, combined

with this microclimate heterogeneity, offers a wide range of possible thermal tolerances in the same ecosystem. This variation, in turn, offers a second potent test of the Thermal Adaptation Hypothesis. Thus far, however, syntheses of Thermal Adaptation have focused on geographic comparisons of 'temperate' vs. 'tropical' environments, potentially missing this within-community variation (for a valuable exception, see Ruibal, 1961).

Here, we test the Thermal Adaptation Hypothesis with 88 ant species from a lowland Panama ant assemblage. Ant colonies are ubiquitous insect societies whose activities ramify throughout ecosystems (Hölldobler & Wilson, 1990; Kaspari, 2000). We test the assumption that smaller ants, like other tiny organisms <<1 g quickly achieve equilibrium temperature (<1 min at 100 mg, Stevenson, 1985; Willmer & Unwin, 1981) and experience boundary layer climates relying mainly on convection while larger ants experience cooler air, but increased radiative heating (Porter & Gates, 1969; Tracy, 1992; Angilletta, 2009). We characterize the microclimate diversity of surfaces in the canopy and understory and test how a 1000-fold range of body size shapes  $CT_{min}$  and  $CT_{max}$  based on the surfaces on which they walk. We test the prediction of Thermal Adaptation that higher  $CT_{max}$ s and broader thermal limits are more common among populations nesting in the warmer, more variable canopy. Finally, we use these data to evaluate the prediction that tropical assemblages of tiny ectotherms are at particular risk to climate change given their low  $CT_{max}$ s relative to projected ambient temperature (Colwell *et al.*, 2008; Diamond *et al.*, 2012b).

### **Materials and methods**

All work was conducted on Barro Colorado Island, (9°9'19" N, 79°50'15" W), a lowland, seasonally wet forest in Lake Gatun of the Panama Canal (Leigh, 1999). BCI has a long history of ant studies (Levings & Franks, 1982; Feener, 1994; Kaspari, 1996) and a fauna of ca. 400 species (Donoso, in preparation).

#### *Quantifying surface temperatures in the canopy and understory*

Between 09:00 and 16:00 hours, December 2011–January 2012, we paired measures of surface temperature and ambient air temperature. We did so in the crowns and understory of 31 individual trees. To obtain temperatures in the canopy, we accessed tree crowns using the single rope technique (Perry, 1978). After descending the tree, we collected understory temperatures within 10 m of the base of the tree. Our goal was to (i) contrast temperatures experienced by ants crawling on surfaces in sun vs. shade (exposure) and on overcast, partly cloudy, or full sun days (cloud cover), and (ii) evaluate the

covariance of surface and air temperature given the importance of the latter in models of global change. We quantified temperature on 2–3 types of surfaces: bark and leaves in the canopy, and bark, leaves, and litter in the understory. The surfaces yielded consistent mean surface temperature in both habitats (Kruskal–Wallis  $P$ 's >0.18) and going forward we report averages for all surfaces in the canopy and in the understory.

To measure temperature, we used a portable IR thermometer (Fluke Corporation, Everett, WA; model 62, resolution 0.2 °C, and an accuracy of 1.5%). Surface temperatures were measured by holding the IR thermometer ca. 5 cm from a surface in full sun; five readings were taken within a 300 cm diameter, each separated by >20 cm. Next, we performed the same measurements on an adjacent shaded surface (e.g., the opposite side of the branch). Finally, we recorded five ambient air temperatures by pointing the thermometer at shaded patches of vegetation >10 m away (See Appendix S1: Comparing analog and IR thermometer measures of air temperature).

#### *Quantifying $CT_{min}$ and $CT_{max}$ in arenas based on surface temperature*

In May–July 2011, ant colonies were collected from ants nesting in the leaf litter and in the twigs and branches of the tropical canopy. We aimed to maximize the number of species sampled at the expense of multiple colonies of the same species, with half the species represented by one colony, and the rest represented by 2–5 replicates. In the lab, voucher specimens were stored in 95% EtOH and others pinned and then identified to species or morphospecies. The remaining ants from the colony were placed in 15 cm diameter plastic petri dishes with cotton balls soaked in distilled water. These colonies were allowed to acclimate in a dark drawer at 27 °C for 24 h. At the end of the trial, ants from the trial and the rest from the colony, including workers, reproductives and brood, were placed in a 1.5 ml microcentrifuge tube which was then frozen at –80 °C. Dry mass of a minor worker, after freeze drying, was recorded for each colony used.

We measured thermal tolerance by observing ants in a close-topped arena of a gridded 15 × 2.5 cm petri dish above a white background and below an HD video camera. To record  $CT_{max}$  and  $CT_{min}$ , we placed 4–6 workers (4 if equi-sized, 5–6 if polymorphic) in the petri dish arena. We varied temperature using a ramping protocol, moving ants from ambient temperature down to  $CT_{min}$  and then up to  $CT_{max}$ . This measure of acute thermal tolerance, when performed over a short period of time, minimizes confounding factors of starvation or desiccation (Terblanche *et al.*, 2011). Moreover, as the core temperature of insects <<1 g equilibrates to ambient temperature in ca. 15–60 s (Willmer & Unwin, 1981; Stevenson, 1985; Huey *et al.*, 1992) – and our ants ranged in mass from 0.01 to 57 mg – the 15–30 min duration of the cooling and heating portions of the experiment should have allowed ants to achieve thermal equilibrium with their surroundings. We verified this with additional experiments using thermocouples implanted in ants (see next section).

At the outset of the experiment, ants were allowed to acclimate for 15 min. Next, the surface temperature of the dish (ca. 27 °C) was measured with an IR thermometer (Mastercool (T), resolution 1 °C). Ants were video recorded for 1 min. To cool the petri dish, a sandwich of waxed paper, 1 cm of high-density foam and frozen cryopacs was slipped under the dish. This served to cool the dish to 20 °C after ca. 15 min. At 20 °C a second one minute video was recorded, monitoring dish surface temperature at 30 and 50 s. To cool to  $CT_{min}$  the foam/wax paper buffer was removed, and the Petri dish reduced to 15 °C. If  $CT_{min}$  was not achieved at 15 °C, we placed a frozen CryoPak above the Petri dish. The ants were observed continuously, the dish was periodically shaken, and when  $CT_{min}$  was achieved (defined as >half of the ants fell over and could no longer right themselves if the dish was gently shaken, or chill coma, Angilletta *et al.*, 2007), the temperature was recorded.

Next, the cryopacs were removed and ants were allowed to return to ambient temperature and normal behavior – grooming and running – a process that took ca. 15–30 min. Those colonies that did not reacclimate were discarded from the trials. We placed the petri dish back on the white base above a commercial heating pad with a 1 cm dense foam buffer. We recorded a second 1-min video at room temperature.

We used the heating pads to warm the petri dish to a surface temperature of 34 °C (ca. 15 min). We recorded ant activity at this temperature for 1 min as above. Finally, to achieve  $CT_{max}$ , we heated the petri dish, sometimes using a second heating pad, and continuously observed the ants as the dish was periodically shaken. The  $CT_{max}$  of the trial was recorded as the temperature when ≥50% of the workers lost the ability to right themselves.

In sum, our protocol always cooled the ants to  $CT_{min}$  before raising them to  $CT_{max}$ . To test if cooling systematically biases the lethal  $CT_{max}$ , we used the above protocol to ramp ants from three species directly to  $CT_{max}$  (Appendix S1: Checking for Ramping Bias). We found no evidence for bias.

#### *Translating IR surface temperatures to air temperatures within the arena boundary layer*

We used a Cole-Parmer Remote-Monitoring Thermocouple Thermometer (advertised accuracy ±2°F between 0 and 60 °C) with type K probe to check air temperatures at the surface of the arena as well as 1, 2, and 3 mm above it. Thermocouples were calibrated by first immersing them in an ice/deionized water slurry and recording temperature every 10 s for 1 min. Then the probes were inserted in four water filled Eppendorf tubes placed in a USA Scientific Thermal-Lok 2-position dry heat bath (advertised accuracy ± 0.2 °C) starting at 30 °C and ramping, in 5-degree increments, up to 60 °C. Again, six temperatures were recorded over 60 s. The thermocouples closely tracked the temperature of the water bath (LS Regression  $r^2 = 0.999$ , water bath temperature,  $C = 0.29 + 1.02 \cdot \text{thermocouple temperature } ^\circ\text{C}$ ,  $n = 8$ ) but slightly underestimated water bath temperatures as temperatures exceeded 40 °C. All thermocouple measurements were thus adjusted using this equation.

To measure the way air temperature changed with height above the petri dish arena we used modeling clay to arrange thermocouples at 0, 1, 2, and 3 cm above the surface arena. The LS Regression of air temperature at 0 mm and IR temperature (0 mm air °C =  $3.17 + 0.91 \cdot \text{IR temperature } ^\circ\text{C}$ ,  $r^2 = 0.99$ ) showed the IR underestimating thermocouple-based air temperature by 3°C at our lowest recorded  $\text{CT}_{\min}$  and overestimating air temperature by 2.5°C at our highest recorded  $\text{CT}_{\max}$ . We adjusted our IR-based values of  $\text{CT}_{\max}$  and  $\text{CT}_{\min}$  accordingly using this equation.

#### Checking surface $\text{CT}_{\max}$ estimates against dry bath estimates

We thus far had measured  $\text{CT}_{\max}$  and  $\text{CT}_{\min}$  since we were interested in constraints on ant activity based on the temperatures of the surface on which the ants were running (and referring to them here as surface  $\text{CT}_{\max}$  and  $\text{CT}_{\min}$ ). In May–June 2014, we checked the repeatability of this measure using another common method for measuring body  $\text{CT}_{\max}$  in tiny ectotherms: a digital dry bath, (USA Scientific Thermal-Lok 2-position dry heat bath, advertised accuracy  $\pm 0.2$  °C). We used the protocols of Diamond and colleagues (e.g., Diamond *et al.*, 2012b), which checked for body  $\text{CT}_{\max}$  by rotating the tubes and checking for a righting response. Confining ants in Eppendorf tubes surrounded by an aluminum dry block also had the advantage of reducing or eliminating boundary layers effects. The ramping protocol – raising temperatures from 36 °C in 2 °C increments every 10 minutes – allowed operative temperature of ants to be more directly estimated. We estimated body  $\text{CT}_{\max}$ s from at least five individuals of 26 species from our original group of 88. They ranged in mass from 0.19 to 15.6 mg, with 11 nesting in the canopy and 15 nesting in the understory. As stated earlier,  $\text{CT}_{\max}$  was recorded when over 50% of ants in a trial lost muscular coordination.

#### Using thermocouples and models to test for time to equilibrium and radiation effects

Animal models – synthetic or taxidermy mounts of the study organism impaled with thermocouples – are an effective way to explore how changing microclimate affects operative temperature (Bakken, 1992). Given the tiny size of most of our species, and our desire to watch them behave, impaling ants with thermocouples was not practical. However, in May and June 2014 we did select a size range of some of our largest ants – *Cephalotes atratus* ( $n = 12$ ), *Dolichoderus bispinosus* ( $n = 12$ ), *Pseudomyrmex gracilis* ( $n = 9$ ), and *Procrystocerus belti* ( $n = 9$ ) – to ask how two components of thermal ecology – time to equilibrium temperature and effect of solar radiation on that temperature – varied with body size.

Workers of the four species were collected, killed by freezing at  $-20$  °C, and weighed. Each midmorning trial was set up in the compound of Barro Colorado Island on a slab of beige concrete. We used four 0.2 mm wide hypodermic thermocouples (Omega model HYP-0) attached to a data logger (Omega 4 channel RDXL4SD, resolution 0.1 °C/

1 °C, accuracy 0.4% + 1 °C) and arranged them thusly: one touching the surface of the concrete, one 1–2 mm above the surface to correspond to the height of a standing ant, and two inserted into a worker ant's thorax (*C. atratus*, *Ps. gracilis*) or gaster (*D. bispinosus*, *Pr. belti*). Two ants (usually of the same species) were recorded simultaneously on separate channels. This setup began in the shade of an umbrella.

At the beginning of a 'sun' trial, the data logger was switched on to record every 2 s, and after ca. 6 s, the umbrella was removed. The trial continued for ca. 120 s, or until solar conditions changed (e.g., by an approaching cloud). Temperatures usually quickly approached ca. 80% of maximum, and then entered a variable phase effected by light breezes. We visually estimated equilibrium temperature of the model as the number of seconds after umbrella removal when the model reached that 80% inflection point. At the beginning of a 'shade' trial, the thermocouple setup was placed under an umbrella and observed until temperatures stabilized, then the data logger was switched on to record every 2 s for 60 s.

To examine the effects of radiation, we compared the average temperature of a model for each sun and shade trial minus the recorded air temperature at its height above the surface.

#### Projected ambient temperatures under warming

To estimate thermal risk for ant populations on BCI with climate warming, we used 2050 and 2080 estimates from three climate models (CCCMA, CSIRO, HADCM3) under two scenarios (A2a and B2a, Ramirez & Jarvis, 2008).

#### Statistics

We used ANOVAS to compare canopy and understory populations, and least square regressions to evaluate the effects of body size and the tradeoff hypothesis. When comparing different measures of environmental temperature of  $\text{CT}_{\max}$ , we used Reduced Major Axis regression. To evaluate any correlates of phylogeny, we constructed a genus-level phylogenetic tree and generated phylogenetically independent contrasts, which were similarly evaluated in the above ANOVAS (See Appendix S1: Analyzing the effect of phylogeny).

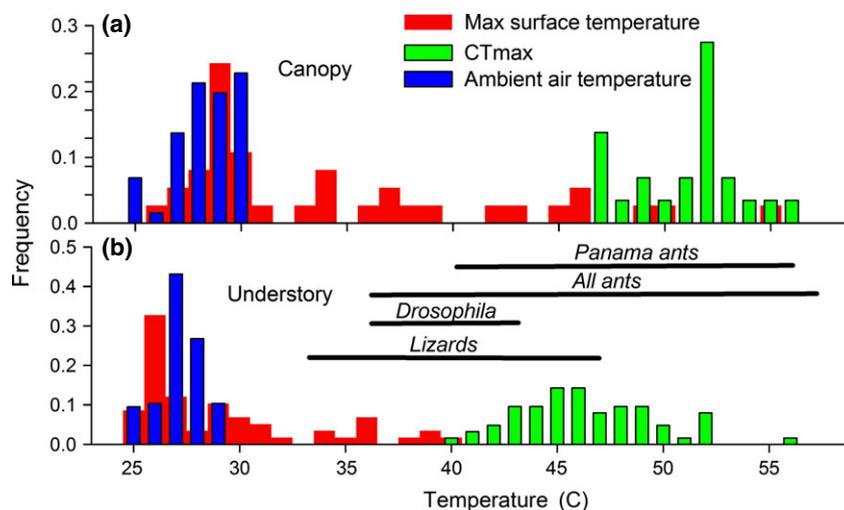
## Results

#### Microclimates of tropical surfaces

Air temperatures averaged only 1 °C warmer in the canopy compared to the understory. In contrast, surfaces exposed to sun averaged about 8 °C warmer in the canopy than the understory. When exposed to the sun, these surfaces were 4 to 10 °C warmer than air temperatures; and coefficients of variation were 4 to 8-fold higher (Table 1, Kruskal–Wallis  $P \ll 0.05$ , as are all other trends reported for the remainder of this section, Figs 1 and S3).

**Table 1** Average temperatures (and Coefficient of Variation) of air and plant surfaces used by worker ants in a Neotropical Forest. Air temperatures were taken following every surface temperature by pointing the infrared thermometer at a shady patch

	Exposure to the Sun		Sky conditions		
	Away	Toward	Overcast	Partly Cloudy	Sunny
Canopy					
Air	27.5 (4.8)	28.2 (2.8)	26.4 (4.1)	28.0 (3.7)	28.4 (2.3)
Surface	27.7 (6.0)	38.4 (16.0)	27.1 (10.0)	31.1 (16.8)	34.0 (20.9)
Understory					
Air	26.8 (4.1)	27.3 (2.6)	25.1 (1.8)	25.1 (1.9)	27.3 (2.9)
Surface	26.6 (3.7)	33.7 (13.0)	25.1 (1.9)	29.1 (13.3)	30.5 (15.8)



**Fig. 1** Distribution of max observed surface temperatures in the canopy and understory of 31 tropical trees vs. the surface temperature at which ants from that habitat died in a laboratory assay. Ambient air temperature from the same habitats is shown for contrast. Horizontal bars contrast range of  $CT_{max}$  in this Panama rainforest, to three meta-analyses, one for 269 populations of ants (Diamond *et al.*, 2012a,b) and one for 94 populations of *Drosophila* (Kellermann *et al.*, 2012) and the third for 70 populations of lizards (Huey *et al.*, 2009).

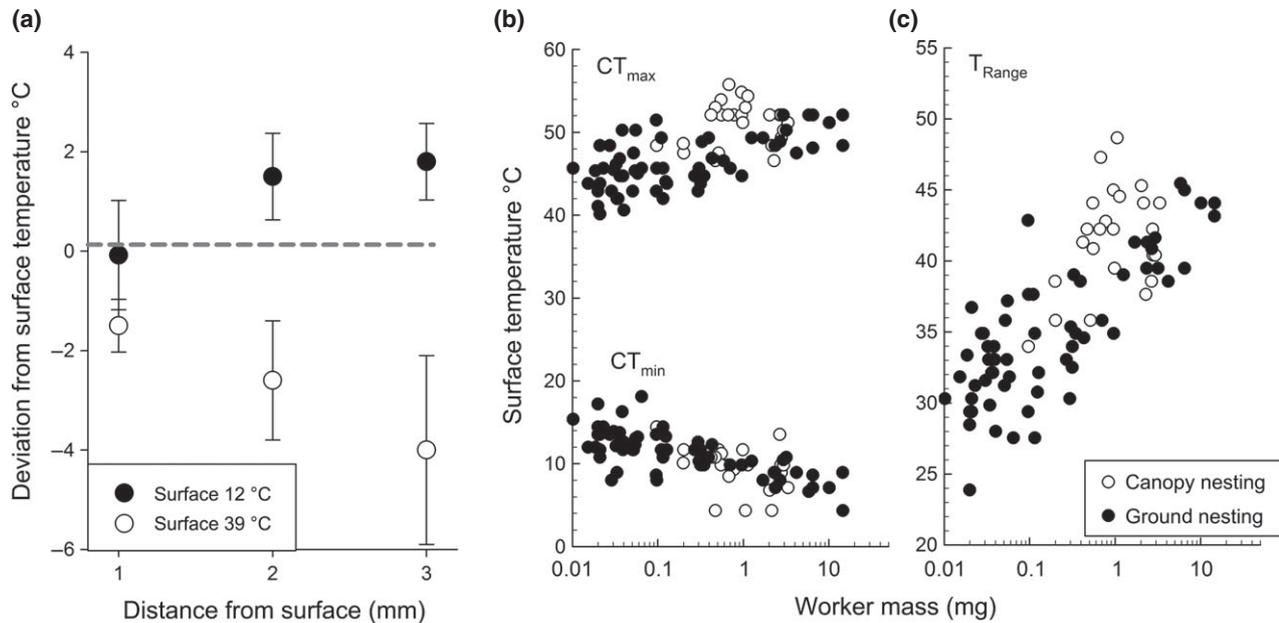
Ambient air temperature was a poor predictor of surface temperatures exposed to sun (Least Square Linear regression: canopy  $r^2 = 0.01$ , understory  $r^2 = 0.12$ ), and was frequently 20 °C cooler (Figure S4). In contrast, ambient air temperature was tightly correlated with the temperatures of shaded surfaces ( $r^2 = 0.64$  and  $0.74$  respectively) and overcast days ( $r^2 = 0.49$  and  $0.87$ , respectively).

#### Thermal limits to activity based on surface temperature

Surface temperatures in the arena differed from air temperatures 1 to 3 mm above the surface (Fig. 2). At a surface temperature of 12.3 °C, air temperature warmed with height as  $T_{air} = -0.85 + 0.97 * mm$ ,  $F_{1, 22} = 16$ ,  $r^2 = 0.43$ ,  $P = 0.0005$ . At surface temperature of 39.2 °C, air temperature cooled as  $T_{air} = -0.23 -$

$1.25 * mm$  ( $F_{1, 10} = 7.7$ ,  $r^2 = 0.43$ ,  $P = 0.0198$ ). Median worker mass varied 1000-fold, from a 0.01 mg *Brachymyrmex* sp. to a 57 mg *Paraponera clavata*. As the latter is ca. 3 mm tall, in still air it could experience operative temperatures four degrees cooler than the smallest ants.

Larger ants survived higher surface temperatures before they lost motor control (Surface  $CT_{max} = 49.2 + 2.9 \log_{10} \text{Mass(mg)}$ ,  $F_{1,86} = 65$ ,  $P < 0.0001$ ,  $r^2 = 0.43$ ), and lower surface temperatures before they grew torpid (Surface  $CT_{min} = 9.5 - 2.22 \log_{10} \text{Mass(mg)}$ ,  $F_{1,85} = 80$ ,  $P < 0.0001$ ,  $r^2 = 0.49$ ) resulting in a 5 °C increase in range of activity for every 10-fold increase in a species' worker mass (Surface  $T_{Range} = 39.7 + 5.1 \log_{10} \text{Mass(mg)}$ ,  $F_{1,85} = 140.8$ ,  $P < 0.0001$ ,  $r^2 = 0.62$ ). All three of these patterns were verified with phylogenetic contrasts ( $P$ 's < 0.05).



**Fig. 2** Boundary layer environments in a petri dish arena and resulting affects of thermal limits to activity as a function of body size. (a) Boundary level effects presented as deviation from temperature at the surface of the (means  $\pm$  1 SD). Below 10 °C, the air above is up to 2 °C warmer; above 40 °C, the air is up to 4 °C cooler. (b) Plot of surface Critical Thermal maximum and minimum and (c) range based on surface temperatures vs. body size for canopy and understory species.

Body size also varies within ant colonies, particularly in 18 taxa from seven genera with dimorphic species. As in the interspecific pattern, majors from polymorphic colonies had thermal ranges 4 °C wider than minor workers (signed rank  $S = 37$ ,  $P = 0.002$ ).

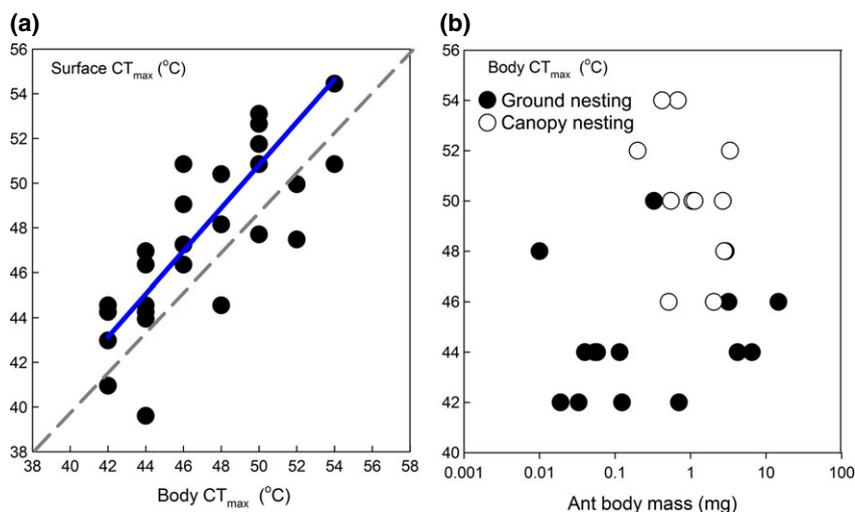
*Habitat effects on surface temperature and thermal limits.* Consistent with the Thermal Adaptation Hypothesis, canopy ants were active at a warmer and broader range of surface temperatures than understory species (Figs 1 and 2). This was achieved in two ways. First, after mass data were log<sub>10</sub>-transformed, canopy species were four times larger, on average, than understory species (0.77 vs. 0.13 mg,  $F_{1,81} = 11.2$ ,  $P < 0.001$ ) and as we have seen, larger ants in this assemblage had broader thermal limits.

Moreover, after accounting for mass, thermal limits were still higher and broader in the canopy. Surface CT<sub>min</sub> failed to vary with habitat (Fig. 2, habitat effect  $P = 0.8$ ). But surface CT<sub>max</sub>, after accounting for body mass (ANCOVA:  $F_{1,84} = 6.7$ ,  $P = 0.01$ ), averaged 3.5 °C higher in canopy than understory species ( $F_{1,84} = 23.0$ ,  $P < 0.0001$ , interaction  $P = 0.30$ , Figs 1 and 2); with a commensurate increase in surface T<sub>range</sub> ( $F_{1,88} = 17.3$ ,  $P < 0.0001$ , interaction  $P = 0.49$ ). Differences among habitats were also found after accounting for phylogeny ( $P < 0.05$ ).

If the range of surface temperatures in Fig. 1 captures the available thermal environment on a sunny day, only 2% of understory species sampled have their surface CT<sub>max</sub> exceeded by the rare 40 °C patch of litter. In contrast, the peak surface temperatures in the canopy episodically exceed the surface CT<sub>max</sub> of all but three of the canopy species; up to 14% overlap the surface CT<sub>max</sub> of at least one species. Not only do canopy ants average higher surface CT<sub>max</sub> than ground species (50 vs. 46 °C), they have a narrower range (9 vs. 15 °C) and coefficient of variation (5.4 vs. 7.0).

#### *Thermal limits based on core temperature from dry baths*

We performed a second test of body size effects on thermal limits by using thermal dry baths, evaluating the dry bath CT<sub>max</sub> for 26 of the original species ranging in size from 0.19 to 15.6 mg. Dry baths provide a uniform thermal environment, eliminating the boundary layer, and are thus more likely to reflect ant core temperatures across the range of body masses tested. An RMA regression found a close correlation between the CT<sub>max</sub>s based on surface temperatures and those based on the thermal dry bath (surface CT<sub>max</sub> = 2.8 + 0.96 dry bath CT<sub>max</sub>, slope  $P < 0.0001$ , intercept NS, Fig. 3a). These CT<sub>max</sub> estimates based on an ant species' operative temperature well predicted its activity based on surface temperature.



**Fig. 3** Comparison of surface  $CT_{max}$  measured from surface temperatures for ants running in a petri dish arena – and experiencing boundary layers – with body  $CT_{max}$  measured from ants trapped in a tube that was heated uniformly in a thermal dry bath. (a) Correlation of  $CT_{max}$ s; gray dashed line is unity; solid line is best fit via an RMA regression. (b) Plot of body  $CT_{max}$  against mass, split into canopy and ground nesting species.

We next used the dry bath  $CT_{max}$  to test for size and habitat effects as stated earlier (Fig. 3b). When the boundary layer effect was removed, canopy nesting ants averaged  $CT_{max}$ s 5 °C higher (ANCOVA  $F_{1,23} = 23.68$ , LSMeans 50.1 vs. 44.7 °C,  $P < 0.0001$ ) but body mass disappeared as a correlate of  $CT_{max}$  ( $F_{1,23} = 0.19$ ,  $P = 0.67$ ).

#### Testing for a tradeoff between $CT_{min}$ and $CT_{max}$

If there is a tradeoff between  $CT_{min}$  and  $CT_{max}$ , the two should be positively correlated. Surface  $CT_{max}$  and  $CT_{min}$  were negatively correlated (Fig. 4a,  $r = -0.45$ ,  $P > 0.0001$ ). Moreover, even after body size effects were removed by least squares regression, the residuals of Surface  $CT_{min}$  and  $CT_{max}$  were uncorrelated ( $CT_{max} = 49.0 + 2.9 * \log_{10}mass - 0.03 * CT_{min}$ ;  $P = 0.87$ , Fig. 4b). Thus, there was no evidence of the predicted tradeoff in thermal tolerances.

#### Using thermocouples and models to test for time to equilibrium and radiation effects

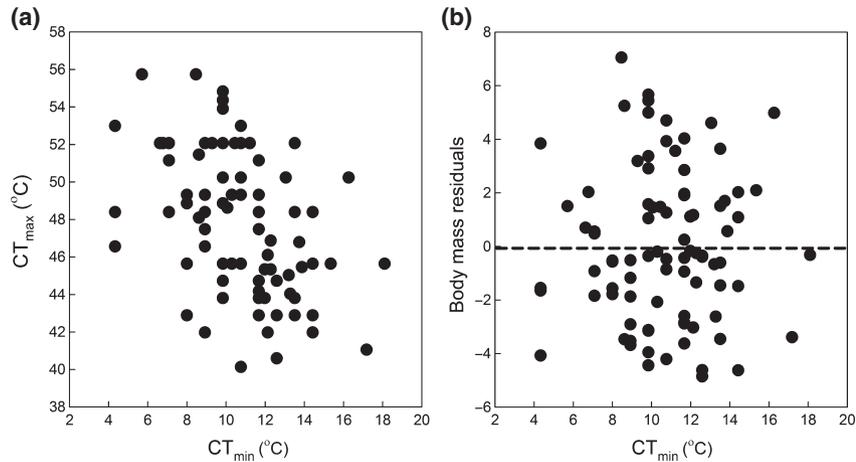
When shaded ants were impaled on thermocouples and exposed to the tropical sun, time to thermal equilibrium increased with body size across the four species (Fig. 5). Time to equilibrium was ca. 10 s for the 1 mg *Procryptocerus belti*, and increased as  $11.4 * Mass^{0.36}$  ( $F_{1,45} = 196.24$ , both parameters  $P < 0.0001$ ,  $r^2 = 0.70$ ). Seventy-two percent of ants in this study were smaller than *P. belti*. Extrapolating, this suggests that the smallest species in this study, from the genus *Brachymyrmex*,

would reach equilibril temperature under similar circumstances in ca. 2 s.

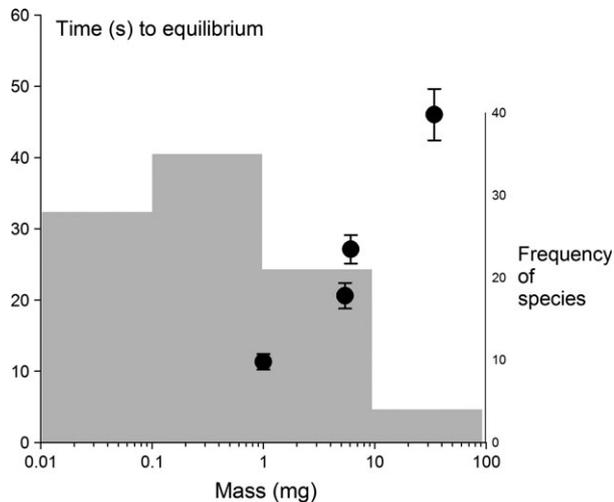
The four species also appeared to absorb/reradiate radiation in different ways (Fig. 6). The difference between model ants and ambient air temperature at the same height varied systematically with body size (ANCOVA  $F_{1,42}$ ,  $P < 0.0001$ ), with a slope that varied between the sun and the shade (sun/shade  $F_{1,42} = 0$ ,  $P = 0.98$ , but interaction  $F_{1,42} = 5.0$ ,  $P = 0.03$ ). In the shade, the difference between model ant and bare thermocouple temperature increased with ant size (difference =  $0.4 + 1.4 * \log_{10}Mass$ ,  $F_{1,21} = 10.5$ ,  $P = 0.04$ ,  $r^2 = 0.33$ ). In the sun, this rate doubled as (difference =  $39.4 + 3.1 * \log_{10}Mass$ ,  $F_{1,21} = 6.2$ ,  $P = 0.02$ ,  $r^2 = 0.23$ ). The two intermediate sized species diverged in the way solar radiation raised operative temperature: the narrower *Pseudomyrmex* showed little evidence of being warmer than the surrounding air in sun or shade, while the hairier and more robust *Dolichoderus* averaged about 4 °C warmer than ambient air in the sun than the shade, in a manner similar to the 10-fold more massive *Cephalotes*.

## Discussion

Tiny ectotherms often experience boundary layer environments that superheat relative to weather station temperatures – the same temperatures used to predict future climates (Potter *et al.*, 2013). The consequence of boundary layers for the thermal tolerances of tiny cursorial organisms like ants become clear when ant tolerances are compared to those of two



**Fig. 4** Test of Thermal Adaptation's prediction of a positive correlation between surface CT<sub>max</sub> and CT<sub>min</sub> (a), and once the effects of body size are removed (b).



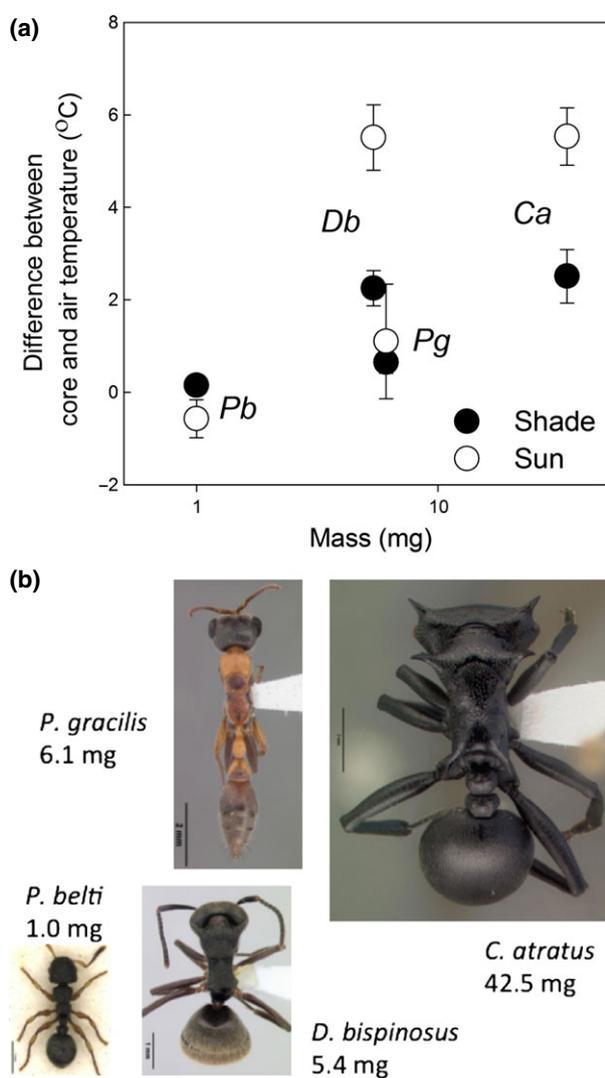
**Fig. 5** Thermocouple studies of taxidermy models of four large common ants from our pool of 88 species (see also Fig. 6). Time to equilibrium temperature (mean and SE) when shading umbrella is removed as a function of body mass. Gray bars represent body size distribution of ants in this study.

other well-studied ectotherm taxa (Fig. 1, Huey *et al.*, 2009; Kellermann *et al.*, 2012). Lizards are large enough to stride above the superheated boundary layer; *Drosophila*, while small, can escape vertically into ambient air. Most ants have neither option. The likely result are maximal thermal tolerances among ant species 10 °C or more higher than those recorded for species of these other taxa. For the tiny cursorial ectotherms that make up much of Earth's biodiversity (May, 1978; Wilson, 1987) life in boundary layers, and the slower speeds afforded by small size, likely select for higher thermal tolerance than larger and/or more volant ectotherms.

#### *The thermal diversity of ant communities*

Tests of the Thermal Adaptation Hypothesis up to now have focused on thermal limits along latitudinal gradients of climate (e.g., Deutsch *et al.*, 2008; Huey *et al.*, 2009). However, the transition from sun to shade found in any closed forest generates a patchwork of surface microclimates spanning at least 10–30 °C (Ruibal, 1961; Willmer & Unwin, 1981; Ferro & Southwick, 1984; Chown & Nicolson, 2004). Moreover, within these forests, treefalls regularly bring the full strength of the sun to the forest floor (Huey *et al.*, 2009). This patchiness within forests generates considerable opportunity for thermal adaptation (Scheffers *et al.*, 2013). It also changes the way microclimates are spatially correlated. Across latitudes, cooler environments are typically more variable (i.e., seasonal). Canopy boundary layers are both warmer and more variable than those in the understory, selecting for temperature generalists, not specialists, in the warmer of the two habitats.

The diversity of microclimates found in any ecosystem thus potentially supports a great diversity of thermal niches for its smallest ectotherms. In our Panama rainforest, we found a range of CT<sub>max</sub>s that accounted for 74% of the known variation (Diamond *et al.*, 2012b) across 269 populations sampled from 0 to 66° North latitude. Such within-community thermal diversity is not unusual: three temperate zone forests in that study each yielded ant populations that collectively represented half the world range of CT<sub>max</sub>; thermal limits from each assemblage overlapped broadly with the others despite average weather station temperatures of 16, 7, and –4 °C. The capacity for boundary layers to superheat—and their poor correlation with ambient temperatures—is one likely reason that the ant communities of Harvard



**Fig. 6** Thermocouple studies of taxidermy model of four large common ants from our pool of 88 species. (above) Difference between the equilibrated temperature of models vs. a naked thermocouple 1–2 mm above the ground at ca. the same height as the ant in the shade vs. the sun. (below) Dorsal photographs of the focal species arranged in the same sequence left to right, and to ca. scale.

Forest and Duke Forest (mean temperature 7 and 16 °C) share a top  $CT_{max}$  of ca. 46 °C, a thermal maximum only 2 degrees higher than that from the Rocky Mountain Biological Lab (mean temperature –4 °C).

Meta-analyses that use latitudinal variation in temperature often predict that tropical ectotherms are at particular extinction risk given the proximity of projected air temperature to  $CT_{max}$  (Colwell *et al.*, 2008; Deutsch *et al.*, 2008; Diamond *et al.*, 2012b; but see Hoffmann *et al.*, 2012; Huey *et al.*, 2009; Kellermann *et al.*, 2012). However, for the tiny organisms that occupy boundary layers, the projected 2–5 °C increase in maximum weather station temperature for Panama

2080 (Stocker *et al.*, 2013) is likely less consequential than factors shaping future light environments (Woods, 2010). For example, decreasing cloud cover brought about by shorter more intense storms (Ipcc, 2007) should generate hotter surfaces. Likewise, increasing tree mortality (Laurance *et al.*, 2013; Silva *et al.*, 2013) should enhance temperatures on the forest floor. Both would likely drive canopy ants with higher  $CT_{max}$ s into the understory (Diamond *et al.*, 2012a). Such seasonal movement already occurs in Panama when leaf fall opens the canopy and warms the litter below (Hahn & Wheeler, 2002). Social insects like ants can also wait out dangerous temperatures in their nest (Cerdá *et al.*, 1998; Diamond *et al.*, 2012a). Such behavioral thermoregulation is increasingly seen as critical to understanding the influence of warming on animal communities (Sunday *et al.*, 2014). However, a decision by the colony to limit worker activity when temperatures approach  $CT_{max}$  may still; results in significant decreases in foraging and territorial defense and, eventually, colony fitness.

#### *Small size and gradient of size breaks tradeoffs*

As ants increased in size, they were more likely to remain active at higher surface temperatures. We suggest this size effect arises from an interaction between the ant and the boundary layer and not due to the kinetics of enzyme membrane or oxygen delivery often proposed for similar phenomenon (Angilletta, 2009). Our main line of evidence is the disappearance of a size effect when  $CT_{max}$  is measured in the boundary layer-free thermal dry baths. The steep gradient of temperature in the petri dish boundary layer (at 40 °C, air 2–3 mm above the surface is 2–4 °C cooler, Fig. 2) suggest large ants were walking above lethal surface temperatures in the same way that they step over gaps that form ravines for small ants (Kaspari & Weiser, 1999). Other aspects of scaling also suggest that larger ectotherms can move about more easily on superheated surfaces. Larger ants often enjoy increased resistance to desiccation (Hood & Tschinkel, 1990; Kaspari, 1993). Larger ants take longer to heat: the largest ant we tested with thermocouples took five times longer than our smallest ant to reach thermal equilibrium when suddenly exposed to the sun (Fig. 5). This higher thermal inertia (Willmer & Unwin, 1981; Stevenson, 1985; Andrew *et al.*, 2013), combined with higher velocity (Peters, 1983; Hurlbert *et al.*, 2008) should allow a large ant to escape a sun fleck or a cool patch that could prove fatal to a smaller ant.

However, as large ants tower above the hottest temperatures generated by the boundary layer, they may also increasingly suffer net heating through solar radiation (Fig. 6). Four taxidermy mounts of common

canopy ants showed modest effects of body size on absorbing radiation in the shade, but when exposed to the tropical sun, the largest ant models experienced a dramatic nearly 6 °C difference from adjacent air temperatures. This increased thermal load is likely exacerbated by the common black coloration of canopy ants (unpublished data), which result from ants balancing the benefits of UV protection and heat stress (Majerus & Majerus, 1998).

#### *Caveats and complexity*

We quantified the short-term physiological state of foraging ants (Angilletta, 2009; Santos *et al.*, 2011). For most ant colonies, only the queen(s) are present through the life of the colony and queens of one litter species have been shown to have narrower thermal maxima than workers (Diamond *et al.*, 2013). More such studies are needed to clarify correlations between worker behavior and colony fitness. But we note the two canopy genera that are most heat tolerant – *Procrystocerus* and *Pseudomyrmex* – nest in thin twigs that likely regularly superheat the entire colony on sunny days. Likewise, thermal adaptation can occur through a variety of mechanisms – from behavior to natural selection and thus on a variety of time scales (Heatwole *et al.*, 1968; Peck *et al.*, 2009; Ribeiro *et al.*, 2013). Quantifying changes in thermal tolerance within populations over time, interannually and seasonally, would be a useful addition to our understanding and prediction of responses to climate change. Social insects – whose colonies generate new cohorts of genetically similar sisters on a regular basis – are a model organism for such studies (e.g., Ribeiro *et al.*, 2013).

Predicting the role of thermal limits in community-wide responses to climate change, especially in the diverse tropics, remains a challenge. First, temperature is but one factor shaping individual and population performance. For example,  $CT_{max}$  is a poorer predictor of range limits of ectotherms than  $CT_{min}$  (Sunday *et al.*, 2012): as one approaches low latitudes and frost-free temperatures, other abiotic effects like desiccation risk (Hood & Tschinkel, 1990; Kellermann *et al.*, 2012) and nutrition (Kasparsi *et al.*, 2008) may shape fitness. The diversity of competitors and predators may also be more effective on a species' warmer border (MacArthur, 1972). Second, we know little of the phenotypic or evolutionary plasticity in thermal tolerance. Tropical populations may harbor more functional and genetic diversity (Adams & Hadly, 2013; Araújo & Costa-Pereira, 2013; Dowle *et al.*, 2013) and have more opportunities for sexual recombination (Rohde, 1992; Kasparsi *et al.*, 2001). The potential thus exists for enhanced plastic and adaptive responses to rapidly changing environments in tropical species. For these reasons,

Earth's diverse tropical forests remain an important laboratory in the study of biotic responses to global change (Overgaard & Sørensen, 2008; Overgaard *et al.*, 2011).

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Amanda Winters and Dana Frederick provided invaluable help in the field. John Longino, and three anonymous reviewers provided helpful comments on an earlier draft. Funding was provided by grants from the NSF (DEB 0842038) to A.D. Kay and M. Kasparsi and (EF 1065844) to M. Kasparsi. AntWeb graciously provided ant images in Figure 6.

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## Supporting Information

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

**Appendix S1. Methods and Results**