


Thermal constraints on foraging of tropical canopy ants

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Abstract Small cursorial ectotherms risk overheating when foraging in the tropical forest canopy, where the surfaces of unshaded tree branches commonly exceed 50 °C. We quantified the heating and subsequent cooling rates of 11 common canopy ant species from Panama and tested the hypothesis that ant workers stop foraging at temperatures consistent with the prevention of overheating. We created hot experimental “sunflecks” on existing foraging trails of four ant species from different clades and spanning a broad range of body size, heating rate, and critical thermal maxima (CT_{max}). Different ant species exhibited very different heating rates in the lab, and these differences did not follow trends predicted by body size alone. Experiments with ant models showed that heating rates are strongly affected by color in addition to body size. Foraging workers of all species showed strong responses to heating and consistently abandoned focal sites between 36 and 44 °C. *Atta colombica* and *Azteca trigona* workers resumed foraging shortly

after heat was removed, but *Cephalotes atratus* and *Dolichoderus bispinosus* workers continued to avoid the heated patch even after >5 min of cooling. Large foraging ants (*C. atratus*) responded slowly to developing thermal extremes, whereas small ants (*A. trigona*) evacuated sunflecks relatively quickly, and at lower estimated body temperatures than when revisiting previously heated patches. The results of this study provide the first field-based insight into how foraging ants respond behaviorally to the heterogeneous thermal landscape of the tropical forest canopy.

Keywords Arboreal · Behavior · Formicidae · Panama · Sunfleck

Introduction

One predicted consequence of global climate change is extinction of tropical animal species specifically because they are already living close to their thermal tolerance limits (Deutsch et al. 2008; Sinervo et al. 2010). However, most estimates of local temperature regimes are based on regional weather data, which are not accurate representations of thermal environments actually experienced by most organisms (Potter et al. 2013), in particular, small animals that traverse highly exposed surfaces when foraging risk exposure to temperature extremes well in excess of their thermal tolerance limits. Under such circumstances, rapid heating disrupts physiological processes and can lead to sudden death (Lowe and Heath 1969; Crawshaw 1979; Denlinger and Yocum 1998), especially among small, wingless, cursorial animals that cannot fly to escape rapidly changing conditions (e.g., lizards, ants). Thus, if foragers do not have sufficient physiological adaptations to tolerate such temperature extremes, they are expected to

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use behavioral adaptations to avoid or flee those conditions (e.g., Gehring and Wehner 1995; Clémencet et al. 2010).

Insects are particularly vulnerable to overheating (and associated desiccation; Hood and Tschinkel 1990; Bujan et al. 2016) due to their relatively large surface area-to-volume ratio. They also are immersed in superheated boundary layers that larger organisms escape via their taller posture (Willmer and Unwin 1981; Stevenson 1985; Hurlbert et al. 2008; Kaspari et al. 2015). Consequently, small cursorial taxa often use in situ behavioral thermoregulation or other behavioral mechanisms to prevent overheating (Porter and Tschinkel 1993; Melville et al. 2001; Penick and Tschinkel 2008; Sinervo et al. 2010). Time spent avoiding extreme thermal conditions limits foraging and other activities that directly affect fitness. Assuming that many animals are driven by competition to maximize their acquisition of limiting resources, the Thermal Constraint Hypothesis predicts that an animal's foraging activity should be closely associated with its thermal tolerance limits (Cerdá et al. 1998; Pereboom and Biesmeijer 2003). Here, we test this hypothesis for tropical canopy ants using field and laboratory experiments to determine how exposure to localized high temperatures affects their foraging behavior.

Tropical forests are patchy light environments with complex vertical stratification (Yanoviak and Kaspari 2000; Madigosky 2004; Scheffers et al. 2013). The forest canopy tends to be hot and dry relative to the understory (Parker 1995), and overhead vegetation (even within tree crowns) creates a spatially and temporally dynamic mosaic of shade and full sunlight (Endler 1993; Meisel 2006). The surfaces and associated boundary layers of exposed portions of branches become superheated, frequently approaching 55 °C (Ruibal 1961; Kaspari et al. 2015), and the sun-exposed top and the shaded underside of a single branch often differ by >10 °C.

Ants are an excellent focal taxon for exploring how tropical ectotherms behaviorally respond to thermal variation. Ants are ubiquitous and play important roles in ecosystem-scale processes (Janzen 1966; Hölldobler and Wilson 1990; Bestelmeyer and Wiens 2003). As cursorial foragers, their ability to escape rapid changes in temperature is critical for survival (Cerdá et al. 1998; Clémencet et al. 2010). Further, the broad range in ant body size (nearly four orders of magnitude), running speed, color, and colony size offers multiple axes for exploring mechanisms of thermotolerance (Kaspari and Weiser 1999; cf. Pereboom and Biesmeijer 2003).

The principal objective of this study was to determine how foraging arboreal ants respond to thermal extremes in the field. We assume that CT_{max} (the body temperature at which an animal loses muscular control; Huey et al. 1992) and heating rate are key traits shaping an ant's behavior when it enters a superheated environment (Cerdá et al.

1998; Hemmings and Andrew 2016). We quantified the heating rates of 11 ant species spanning three orders of magnitude in mass from a lowland forest of Panama, and examined how four of those species respond to sudden thermal extremes (e.g., experimental “sunflecks”) in the field. To experimentally test the fundamental thermodynamics underlying the Thermal Constraint Hypothesis, we chose two ant characteristics that should affect their heating rates in predictable ways: color and size. Specifically, we expected heating rate to decrease with increasing mass, and to be higher for dark-colored versus light-colored ants and experimental ant models. We also predicted that as temperature increases, different ant species sequentially cease using the sunfleck in a manner correlated with their CT_{max} . Finally, we predicted that ants with higher heating rates, which are more likely to achieve CT_{max} in a sunfleck, will return to superheated patches at lower temperatures.

Materials and methods

Study site

This research was conducted on Barro Colorado Island, Panama (09.15°N, 79.85°W), a seasonally moist forest managed by the Smithsonian Tropical Research Institute (hereafter, BCI). All data were collected during the early dry season (January–February) of 2015. Additional information about BCI and the region is available elsewhere (Leigh et al. 1996).

Ant heating rates in an artificial sunfleck

We collected >30 foraging workers from at least three different colonies of 11 ant species on BCI and killed them by freezing at −20 °C for >1 h. To quantify interspecific differences in heating rate, we placed ants on a white ceramic tile in the laboratory and heated them under a 125 W UVA/UVB mercury vapor heat lamp (Solar Glo, ExoTerra, Mansfield, MA). Ants were positioned on the tile in a normal (dorsal-side up) walking posture, but at least partial contact between the ventral surface of the ant bodies and the tile was unavoidable. Although not perfect, we believe this approach provided a reasonably good approximation of the thermal dynamics within ant bodies (Hemmings and Andrew 2016). Each ant was thawed for 30 min and weighed to the nearest 0.1 mg before heating.

We inserted a mini-hypodermic thermocouple probe (Omega HYP-0; Stamford, CT) into the thorax of large ants via the cervical foramen following decapitation. For smaller ants (*Azteca trigona*, *Procryptocerus belti*, *Pseudomyrmex elongatus*, and *Pseudomyrmex gracilis*) we inserted the probe into the gaster via the rectum. The

probes were attached to a four-channel data logger (Omega model RDXL4SD), which allowed us to simultaneously heat three ants and one control probe per trial. The control probe was secured above the surface of the tile to match the height of the estimated center of mass of the focal ant species. The logger recorded data every 2 s for one minute and was started a few seconds before the lamp was turned on. In all, we heated 309 ants in 103 trials; only one species was heated in each trial. Preliminary tests with 11 *Camponotus* spp. workers showed no difference in heating rate or maximum recorded temperature between probes inserted into the gaster and thorax of the same ants (paired *t*-tests: $t < 0.59$, $df = 10$, $p > 0.57$).

In each experimental trial, all four probes rapidly heated to maximum temperature within 20 s, and then heated at a much slower rate during the subsequent 40 s (Supplemental Fig. S1). Given that ants are unlikely to remain in a superheated location in a natural setting for more than 20 s (see Results), we focused only on this initial heating phase for analysis. Specifically, we used two observed variables to calculate a third variable to quantify the thermal dynamics of the ant bodies, as follows: T_0 = probe temperature ($^{\circ}\text{C}$) before the heat lamp was turned on; T_{20} = probe temperature ($^{\circ}\text{C}$) observed after 20 s of continuous heating; and H_a = the heating rate ($^{\circ}\text{C s}^{-1}$) of the probe inside an ant, calculated as $(T_{20} - T_0)/20$. We used the same approach to calculate a fourth variable, H_c = the heating rate of the control probe. T_0 and H_c did not differ among species (ANOVA; $F_{10,90} < 0.52$, $P > 0.88$ in both cases), thus we focused solely on H_a as the response variable. We similarly measured the cooling rates ($^{\circ}\text{C s}^{-1}$) of the ant bodies (C_a) and control probes (C_c) by measuring the change in temperature over 10 s beginning the moment the lamp was turned off.

Effects of body size and color on heating rates

To provide a basis for understanding how ant body size and color affect their thermal dynamics (e.g., Pereboom and Biesmeijer 2003; Azócar et al. 2016), we warmed a series of model ants (small spheres of modeling clay; Polyform Products, Elk Grove Village, IL) using the heat lamp protocol described above. Specifically, we created 38 black clay spheres ranging in mass from 1 to 250 mg, and 38 white spheres spanning the same size range. Thermocouple probes were inserted into the spheres, which were then heated under the lamp for >60 s. We used the heating rate ($^{\circ}\text{C s}^{-1}$) during the first 20 s of heat lamp exposure as the datum for each ant model.

We measured the effects of ant color on heating rate in the field as a corollary to the clay models measured in the laboratory. We collected, killed, and weighed 20

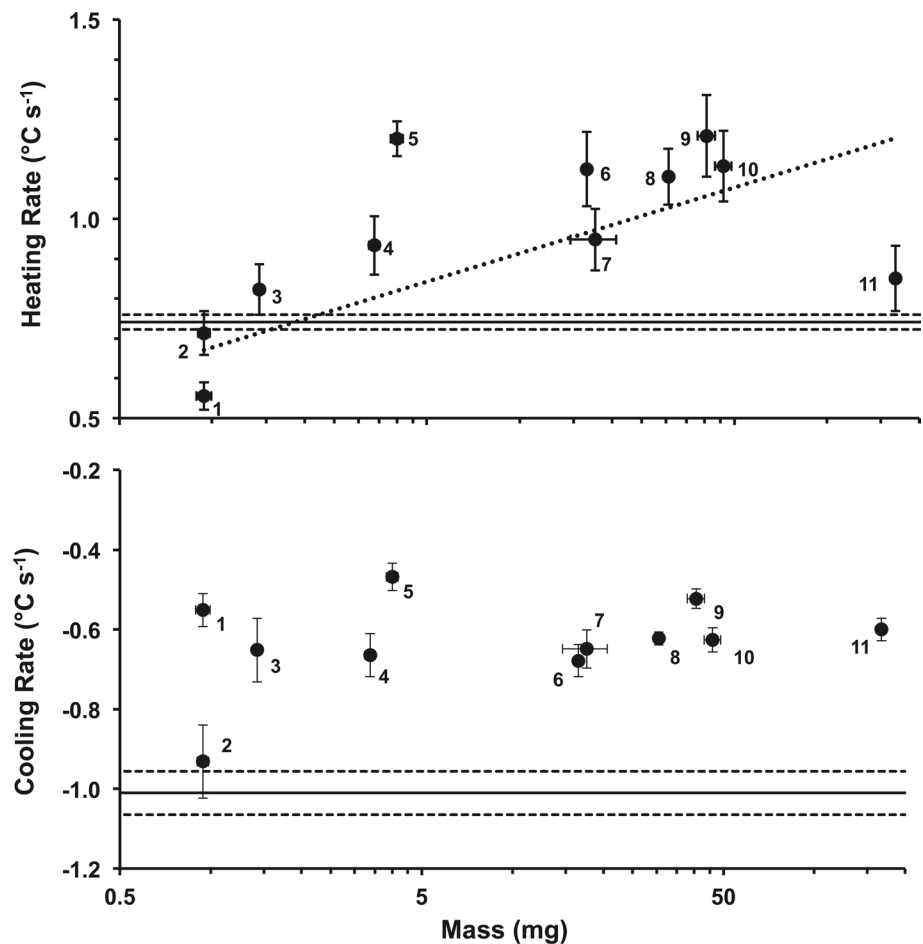
Cephalotes atratus workers from multiple colonies as described above. We used *C. atratus* for this experiment because the workers are continuously polymorphic, span a broad range of body size, and are easy to collect and handle. We painted the dorsal surface of each worker either black ($n = 10$) or white ($n = 10$) with fingernail polish (Laboratorios Darosa, S. A., Guatemala). Workers of *C. atratus* are naturally black in color; the black paint served as a control for paint effects. We inserted a thermocouple probe into the thorax of one black and one white ant as described above. The probes were connected to the data logger, and the ants were arranged ca. 10 cm apart on a beige concrete slab under the shade of an umbrella (see Kaspari et al. 2015). The umbrella was removed to expose the ants to full sun for >40 s. We used the heating rate ($^{\circ}\text{C s}^{-1}$) during the first 20 s of full sun exposure as the datum for each ant.

Testing Thermal Constraints: associating behavior with CT_{max} and heating rate

To determine how thermal extremes in the forest canopy affect ant foraging behaviors, we focused on four common arboreal species (*Cephalotes atratus*, *Atta colombica*, *Dolichoderus bispinosus*, and *Azteca trigona*). We chose these species because they represented the full range of H_a values measured as described above; *C. atratus* and *A. trigona* had the maximum and minimum H_a respectively, whereas the other two species were intermediate (Fig. 1). Moreover, these species span a broad range of body size, are common around the lab clearing on BCI, and are easily recognized. For each of the four focal species, we located foraging trails on tree trunks, branches, or stems of woody vines (lianas), ca. 1 m above the ground. Trail selection was limited by access to electricity for powering the heat lamp, thus some ($<20\%$) of the focal trails were on the ground.

To record ant behavior on each foraging trail, we isolated a specific trail section that could be observed within the field of view of a digital camera (Canon ELPH 115 PowerShot) mounted on a small tripod. The length of the focal section was delimited with metal pins placed 4 cm apart. Two additional pins were offset 1 cm in the foreground and background of the focal plane when the trail occurred on broader surfaces (e.g., on leaf litter or tree trunks; Fig. 2). We measured temperature at the center of the focal section with thermocouple probes (Omega model HYP-1) connected to a four-channel data logger recording at 2 s intervals as described above. We used a heat lamp mounted on a tripod above the focal trail section to generate an artificial sunfleck (Fig. 2). We adjusted the distance of the lamp from the surface plane such that the temperature of the focal section was raised from ambient to 55°C ,

Fig. 1 *Top panel* the average (\pm SE) heating rate (H_a , $^{\circ}\text{C s}^{-1}$) versus average mass (mg) for 11 ant species over 20 s of exposure to a heat lamp. The regression (*dotted line*) is included to illustrate the statistical trend, but does not account for phylogeny. *Bottom panel* the cooling rate (C_a , $^{\circ}\text{C s}^{-1}$) for the same ants during 10 s after the lamp was turned off. $N > 30$ for each species. Numbers next to the plotted means indicate ant species as follows: 1 *Azteca trigona*, 2 *Pseudomyrmex elongatus*, 3 *Procrystocerus belti*, 4 *Dolichoderus bispinosus*, 5 *Pseudomyrmex gracilis*, 6 *Odontomachus bauri*, 7 *Atta colombica*, 8 *Neoponera foetida*, 9 *Cephalotes atratus*, 10 *Camponotus sericeiventris*, 11 *Paraponera clavata*. The horizontal line in each plot indicates average control probe heating or cooling rate, respectively (*dashed lines* are \pm SE)



and then made minor adjustments to maintain the temperature of the focal section at ca. 55°C for 5 min. We chose 55°C as a target temperature because it corresponds to maximum branch temperatures observed in our previous work at this site (Kaspari et al. 2015).

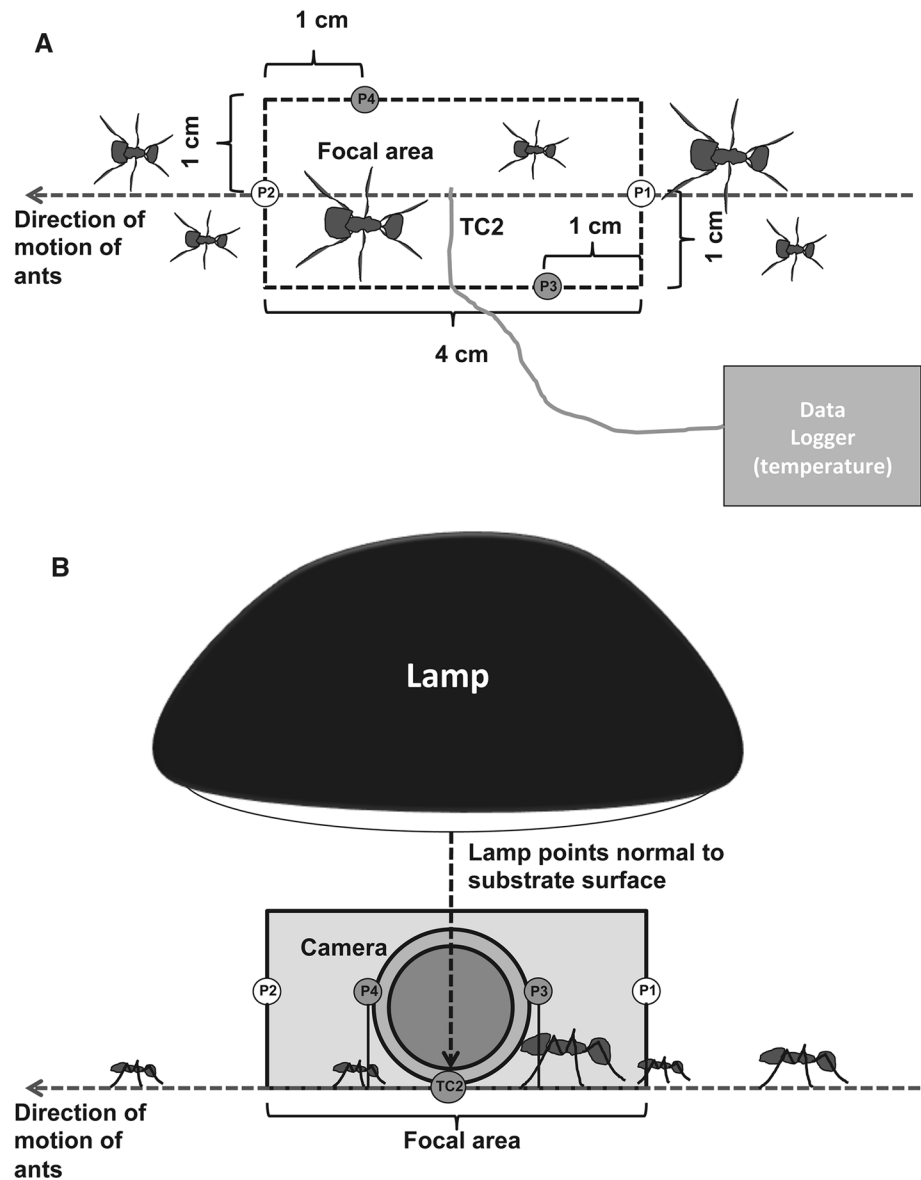
To mimic the effects of a passing cloud or sunfleck, we recorded ant behavior in the focal section for 5 min before the lamp was turned on, during the 5 min heating cycle, and for 5 min after the lamp was turned off. We repeated this heating regime five times in three separate colonies per species, for a total of 60 trials (4 species \times 15 trials each). We excluded several videos that were not of sufficient quality to generate behavioral data or trials that included too few ants (i.e., <5 foragers) for quantification. This resulted in a total of 14 trials for *A. colombica* and *D. bispinosus*, eight for *A. trigona*, and 15 for *C. atratus*.

During each phase of the three-part heating regime, we quantified ant abundance within the focal site once per minute, resulting in 15 abundance measurements per video (i.e., five observations before the lamp was turned on, five during the heating phase, and five after the lamp was turned off). We also quantified two distinct behaviors displayed by workers of all species: *reverse* and *revisit*. *Reverse* behavior

occurred only during the heating cycle, and was characterized by an ant entering the focal section, reversing course, and then exiting the section. *Revisit* behavior was limited to the post-heating phase of the experiment and was defined as an ant reentering the focal section. Thus, *reverse* is the cessation of foraging due to thermal limitation, and *revisit* is the resumption of foraging as the substrate cools to tolerable temperatures. We recorded the temperature at which these behaviors occurred the first five times in each trial.

Finally, to link the heating rates of ants measured in the laboratory with their CT_{max} and subsequent behavior in the field, we determined the average time required for the four focal species to warm to their respective CT_{max} values (obtained from dry bath heating in a separate study; Kaspari et al. 2015). We then estimated the internal temperatures experienced at the moment of *reverse* among the focal species and compared those estimates to their respective CT_{max} values. We generated these estimates by assuming the initial internal temperature of an ant was equal to ambient temperature prior to entering the focal frame. We then multiplied the species-specific heating rate by the number of seconds an ant was in the focal frame before reversing course.

Fig. 2 Experimental setup for field trials, from above (a) and from the side (b). TC = thermocouple placed in center of the focal section under the lamp, P1 entrance point of ants to the focal section (4 cm total length). When substrates were >5 cm wide, P3 was the depth of focal section furthest from the camera, and P4 was the depth of the focal section closest to the camera (2 cm total width)



Statistical analyses

We compared heating rates (H_a) and cooling rates (C_a) among the 11 ant species using analysis of covariance (ANCOVA) with ant mass as the covariate. We similarly used ANCOVA to compare the heating rates of black and white ant models, and the black- and white-painted *C. atratus* workers, respectively. This study focused on relatively few species that are phylogenetically diverse within ants, thus we did not expect the results to be strongly biased by phylogenetic effects. However, we tested for a phylogenetic signal in the distribution of H_a and C_a using the K-statistic (Blomberg et al. 2003) calculated with the *picante* package in R (R Development Core Team 2014). We used the phylogeny of Moreau and Bell (2013) pruned to 11 branches

representing the clades occupied by the species used in this study.

To determine if rapid heating affected ant abundance on foraging trails, we used a mixed model ANOVA and post hoc Tukey HSD with heat treatment (preheat, heat, post-heat) and species as fixed effects. The species \times treatment interaction was significant, thus we tested for differences among treatments for each focal species separately. To test the Thermal Constraint Hypothesis (i.e., that foraging behaviors are constrained by thermal tolerance limits), we compared the average temperature at which *reverse* and *revisit* behaviors occurred within each species using paired *t* tests. We then compared whether the mean temperatures for each of these two behaviors differed across the four focal species using mixed model ANOVAs. Estimated

internal body temperatures at the moment that *reverse* and *revisit* behaviors occurred were compared among species with ANOVA.

We used Bonferroni-adjusted α to correct for multiplicity. Data were transformed as needed to meet test assumptions. All statistical analyses were performed in R version 3.2.3 (R Core Team 2014) or JMP version 11.1.1 (SAS Institute 2009).

Results

Heating and cooling rates differ among ant species

Ant bodies used in the laboratory trials heated very quickly; in all species except *Azteca trigona*, probe temperatures were >50 °C after 20 s of heating. Average T_{20} for the 11 ant species ranged from 42.6 °C to 55.2 °C. Heating rate (H_a) differed among ant species (ANCOVA: $F_{10,291} = 9.43$, $P < 0.0001$), and larger ants heated more quickly than smaller ants ($F_{1,291} = 6.11$, $P = 0.014$; mass \times species interaction $P = 0.38$); *Azteca trigona* and *C. atratus* had the slowest and fastest H_a , respectively (Fig. 1). Assuming that the control probe temperatures closely approximate ambient air temperatures, one species (*A. trigona*) heated more slowly than the surrounding air, three species (*Pseudomyrmex elongatus*, *Procrystocerus belti*, and *Paraponera clavata*) heated at a rates similar to ambient, and the remaining six species heated significantly faster than ambient (Tukey HSD tests; Fig. 1). The average cooling rate of *Pseudomyrmex elongatus* was significantly faster than *Azteca trigona*, *Procrystocerus belti*, and *Pseudomyrmex gracilis*; otherwise, cooling rates were similar among the focal species (ANCOVA: $F_{10,163} = 2.65$, $P = 0.005$; mass \times species interaction $P = 0.45$; Fig. 1). Cooling rates showed no relationship with ant mass ($F_{1,163} = 0.72$, $P = 0.40$). There was no phylogenetic signal in ant heating rates ($K = 0.23$) or cooling rates ($K = 0.12$).

Body size and color affect heating rates of ants and ant models

The distribution of H_a in Fig. 1 suggests that larger ant bodies heated faster than smaller ant bodies, especially for species smaller than *Paraponera clavata*, which is the largest ant in the BCI forest. This pattern was marginally significant (regression: $F_{1,281} = 6.13$, $P = 0.013$, $\alpha = 0.025$; $R^2 = 0.09$), but we caution that the analysis was conducted post hoc and does not account for phylogenetic effects. By contrast, the heating rates of clay models and of *C. atratus* workers decreased linearly with mass (Figs. 3, 4).

Black clay spheres had higher heating rates than white spheres across a broad range of mass (ANCOVA

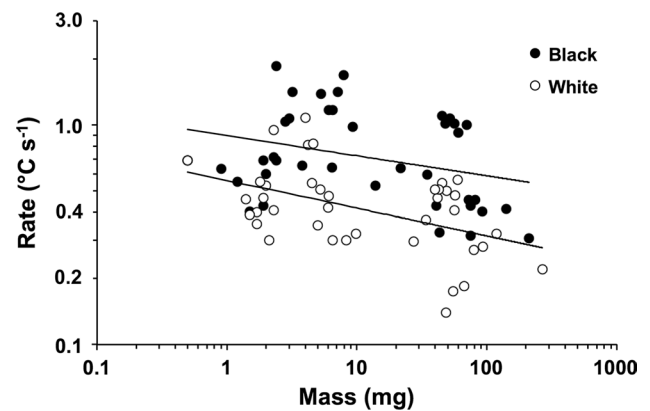


Fig. 3 The heating rate ($^{\circ}\text{C s}^{-1}$) of white ($n = 38$) and black ($n = 38$) model ants (clay spheres) as a function of their mass (mg) during 20 s of exposure to a heat lamp in the laboratory. The linear relationships are white spheres, Rate = $0.56 \text{ Mass}^{-0.126}$, $R^2 = 0.215$; black spheres, Rate = $0.90 \text{ Mass}^{-0.091}$, $R^2 = 0.095$

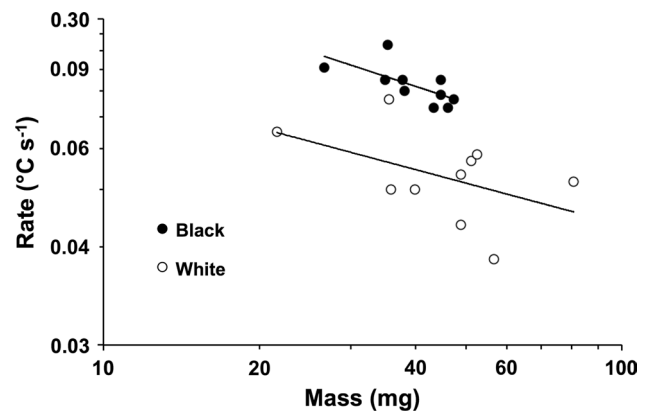


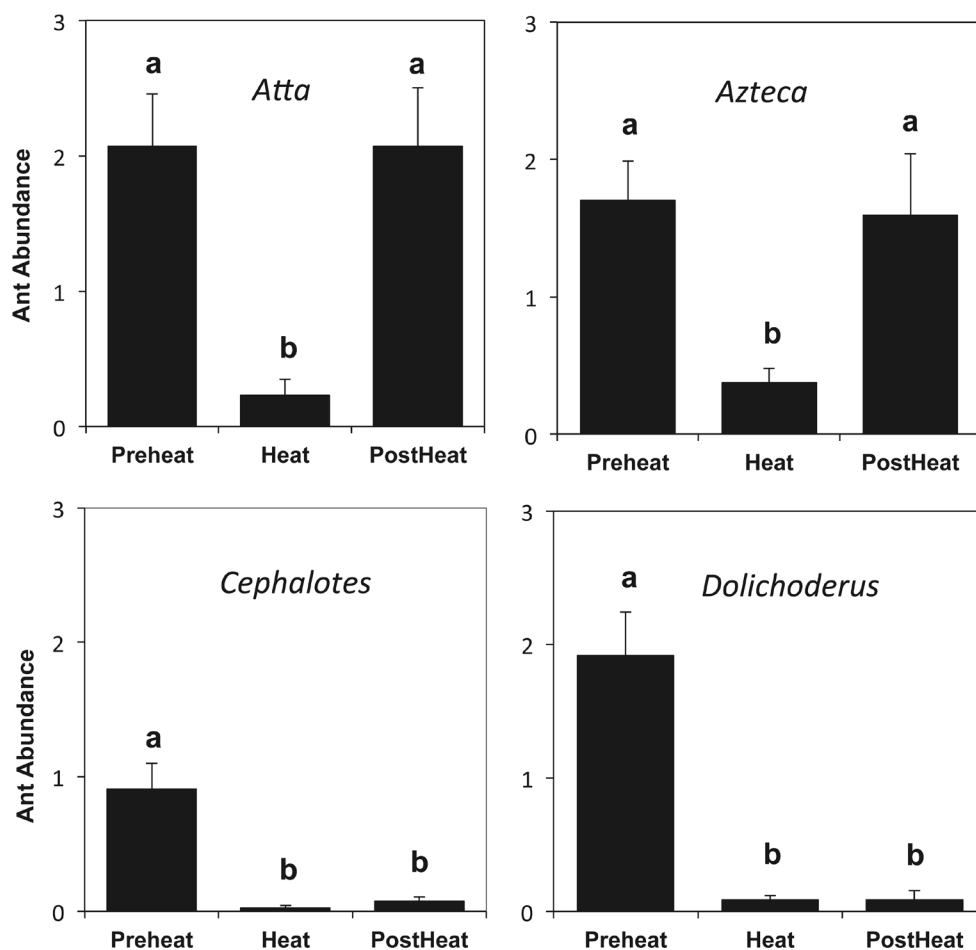
Fig. 4 The heating rate ($^{\circ}\text{C s}^{-1}$) of white-painted ($n = 10$) and black-painted ($n = 10$) *Cephalotes atratus* workers as a function of their mass (mg) during 20 s of exposure to full sun in the laboratory clearing. The linear relationships are white ants, Rate = $0.50 \text{ Mass}^{-0.426}$, $R^2 = 0.222$; black ants, Rate = $0.39 \text{ Mass}^{-0.219}$, $R^2 = 0.454$

$F_{2,75} = 19.48$, $P < 0.0001$; mass \times color $P = 0.99$; Fig. 3). Likewise, *C. atratus* workers painted black had higher heating rates during full sun exposure than did white-painted *C. atratus* workers across a similar size range ($F_{2,17} = 24.94$, $P < 0.0001$; mass \times color $P = 0.84$; Fig. 4). The black-painted workers also warmed to significantly higher average (± 1 SD) maximum temperatures (48.7 °C ± 2.06) than the white-painted workers (44.2 °C ± 1.96) following >40 s of continuous sun exposure (Paired t test: $t = 6.68$, $df = 9$, $P < 0.0001$).

Effects of extreme heat on ant foraging behavior

The application of heat to foraging trails significantly reduced the number of foragers present in all four focal

Fig. 5 Average (\pm SD) ant abundance observed on focal sections of foraging trails during different phases of the heat treatments. Within each species, similar letters indicate means that do not differ based on Tukey HSD tests



ant species (Fig. 5, $F > 24.40$, $P < 0.001$ in all tests). However, ant behavior after the heat was removed differed among species. Specifically, the abundance of *A. colombica* and *A. trigona* foragers did not differ between the pre- and post-heating phases, whereas *C. atratus* and *D. bispinosus* worker abundance remained lower than pre-heating abundance even after the focal patch of trail cooled for 5 min (Fig. 5). Thus, in contrast to *A. colombica* and *A. trigona*, the appearance of a sunfleck served as a persistent deterrent to foraging in *C. atratus* and *D. bispinosus*.

We tested the prediction that the body temperatures at which ants choose to cease using a sunfleck (*reverse*) match the temperatures at which they choose to return to that portion of trail after the sunfleck is removed (*revisit*). Those two temperatures failed to differ between *Atta colombica* and *Dolichoderus bispinosus* (Fig. 6). However, the average *reverse* temperature for *A. trigona* workers was 3.3 ± 1.1 °C lower than their *revisit* temperature ($t = 3.05$, $df = 39$, $P = 0.004$; Fig. 6). The opposite pattern occurred for *C. atratus* workers: their *reverse* temperature averaged 3.3 ± 1.5 °C higher than their *revisit* temperature

($t = 2.25$, $df = 27$, $P = 0.033$; Fig. 6), suggesting that once immersed in a sunfleck, they continue foraging at temperatures that they would otherwise avoid. There was no difference in the artificial sunfleck temperature at which all four focal ant species exhibited *reverse* behavior ($F_{3,8.12} = 1.40$, $P = 0.31$; Supplemental Fig. S2).

Average cooling rates were similar for all four focal species (Fig. 1), and the average temperature at which they exhibited *reverse* or *revisit* behaviors was well below their CT_{max} value (Fig. 6). The estimated internal body temperature at *reverse* (estimated from H_a values; Fig. 1) was significantly lower in *A. trigona* than the other three species ($F_{3,53} = 6.25$, $P = 0.001$; Supplemental Fig. S3). *Azteca trigona* workers also required more time to heat to CT_{max} than did the other focal ants ($F_{3,56} = 37.2$; $P < 0.0001$; Fig. 7), which did not differ from each other ($P > 0.45$ in all tests; Fig. 7).

Although not quantified, all species exhibited faster and relatively erratic motion during the five minute heating period prior to *reverse* behavior. *Atta colombica* workers also consistently dropped the leaves or flowers they were carrying at 52.3 ± 0.79 °C. Worker

Fig. 6 Average (\pm SD) temperature at which foragers of *Atta colombica*, *Azteca trigona*, *Cephalotes atratus*, and *Dolichoderus bispinosus* turned away to exit the focal site (Reverse) and returned to the focal site (Revisit). Asterisks denote significant differences in within-species paired t-tests (* $P < 0.05$; ** $P < 0.005$; ns = $P > 0.05$). Horizontal dashed lines indicate CT_{max} values for each focal species

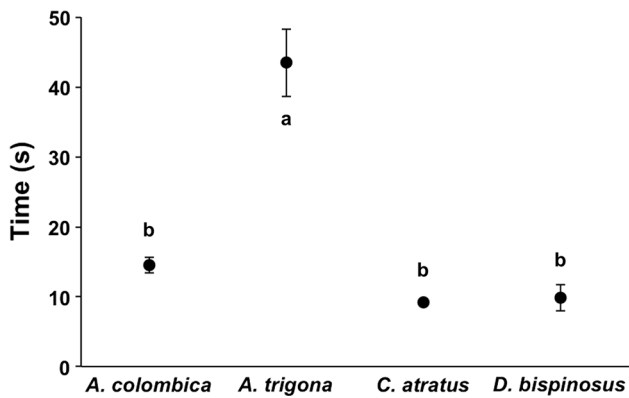
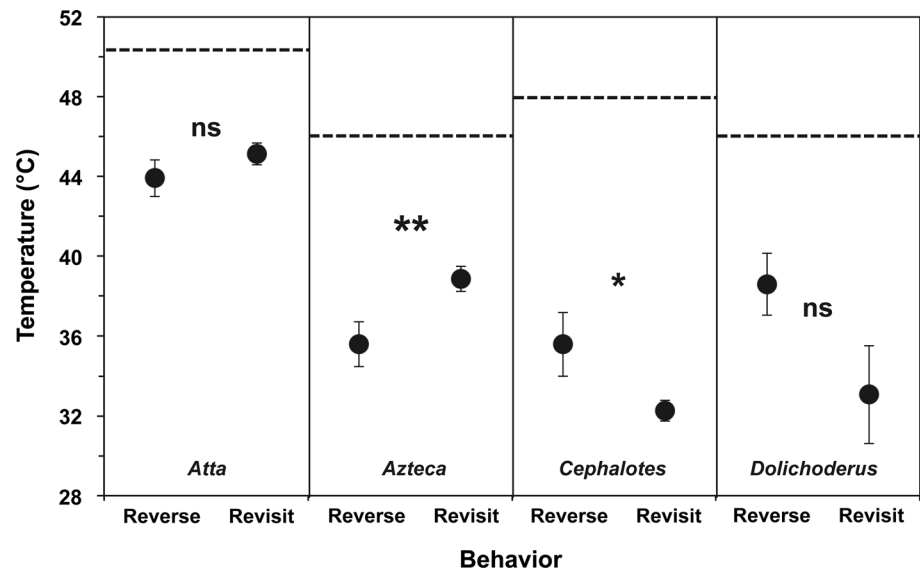


Fig. 7 Average (\pm SD) time required for bodies of *Atta colombica*, *Azteca trigona*, *Cephalotes atratus*, and *Dolichoderus bispinosus* workers to warm to their respective CT_{max} values under the heat of a lamp in the laboratory. Similar letters indicate means that do not differ based on Tukey HSD tests

mortality occasionally occurred in *D. bispinosus* and *A. trigona* workers during the experiment, presumably from overheating.

Discussion

Temperature extremes present an important and often stochastic risk for foraging animals, especially small cursorial ectotherms like ants (Cerdá et al. 1998); however, in situ studies of ant behavioral responses to extreme heat are few and mainly focus on desert ants (e.g., Clémentet et al. 2010; Sommer and Wehner 2012). Here, we show that the foraging behaviors of common Neotropical forest canopy ants support the Thermal Constraint Hypothesis.

Specifically, the spatially and temporally stochastic distribution of superheated patches of leaves and branches (i.e., sunflecks) in the forest canopy disrupts the foraging activities of arboreal ants; they abandoned or avoided foraging at temperatures 5–10 °C below their thermal tolerance limits. This outcome indicates, as in other studies (e.g., Pereboom and Biesmeijer 2003), that thermal extremes pose a significant risk to small ectotherms when foraging. Although stereotypical responses of ants to high temperatures are well documented (Talbot 1943; Marsh 1985; Kaspari et al. 2015, 2016), the relevance of these responses under field conditions was unknown.

The heating rates of the 11 species of tropical canopy ant measured in this study differed from previous studies of the effect of insect body size on thermal properties (Willmer and Unwin 1981; Stevenson 1985). Specifically, the distribution of H_a in Fig. 1 suggests that ant heating rates increase with body mass (with the exception of *Paraponera clavata*). This result is contrary to the prediction (from basic physics of diffusion and conductive heating; e.g., Vogel 2013) that larger bodies will heat more slowly, as shown in other ectotherms (e.g., Pereboom and Biesmeijer 2003; Azócar et al. 2016), and by the clay models and painted *C. atratus* workers measured in this study. In particular, the very slow heating rate of *Azteca trigona* (slower than the surrounding air) was unexpected. However, small ants should desiccate somewhat faster than large ants (Hood and Tschinkel 1990), and workers of *A. trigona* are exceptionally prone to desiccation relative to their CT_{max} (Bujan et al. 2016), suggesting that rapid water loss from their thin exoskeleton or from the probe entry point during heating had a cooling effect. Indeed, average time to desiccation (from Bujan et al. 2016) and average heating rate were highly positively correlated for the 11

focal species in this study (Pearson $r = 0.825$). We did not measure ant mass at the end of the heating trials, but such data would potentially reveal an evaporative cooling effect. The observed increase in heating rate with ant mass also appears to contradict other studies showing that larger ants heat to equilibrium temperatures more slowly than smaller ants (Kaspari et al. 2015); however, this is not a straightforward comparison because larger ants in both studies heated to higher maximum equilibrium temperatures than smaller ants.

Ultimately, the results of this and other studies (Kaspari et al. 2015; Bujan et al. 2016; Hemmings and Andrew 2016) suggest that factors other than body size contribute significantly to interspecific variation in ant thermal dynamics, as with ant desiccation rates (Hood and Tschinkel 1990; Bujan et al. 2016). Relevant factors could include exoskeleton thickness, color, and reflectance (e.g., Willmer and Unwin 1981; Pereboom and Biesmeijer 2003), the size of spiracles, the presence of pilosity, or the stature of the ant (i.e., the distance of the thorax above the surface, which affects its position in the thermal boundary layer; Sommer and Wehner 2012; Kaspari et al. 2015). Exploring all of these factors was beyond the scope of this study, but the strong association with desiccation results described above suggest that interspecific differences in exoskeletal or cuticular thickness is a key variable. Likewise, the consistent difference in heating rates of black and white clay models, and the ca. 5 °C average difference in maximum temperature between white- and black-painted *C. atratus* suggest that color is relevant to radiative heating at the scale of ants (as in tropical stingless bees; Pereboom and Biesmeijer 2003). In combination, these results suggest that the relatively slow heating rates of three of the large ant species examined in this study are either due to their thick, relatively bare exoskeletons (*C. atratus* and *N. foetida*), or thin exoskeleton covered with dense reflective pilosity (*C. sericeiventris*). Further experimental manipulations (e.g., of color or pilosity; Shi et al. 2015) are needed to clarify the role of such traits on ant thermal dynamics.

The field-based behavioral results of this study also did not support expectations derived from laboratory-based thermal tolerance experiments (Kaspari et al. 2015). Despite representing different clades and body sizes, the four focal species of ants abandoned sunflecks at approximately the same temperature. Likewise, the return temperatures did not follow trends expected by body size; the largest ant species (*C. atratus*) returned to the focal site at the lowest temperature, while a mid-sized ant (*A. colombica*) returned at the highest temperature. As explained above, the observed differences in behavioral responses to heat could be due to coloration (Majerus and Majerus 1998; Kaspari et al. 2015), phylogenetic constraints (Cerdá et al. 1998; Diamond et al. 2012; Hoffmann et al. 2013), colony-level

adaptations such as foraging mode (Ruano et al. 1999), or the persistence of trail pheromones at different temperatures (van Oudenhove et al. 2012). More extensive surveys of ant behavioral responses to temperature across diverse phylogenetic and morphological gradients are necessary to understand the species-specific and intraspecific variation in these behavioral responses in the field.

The simplest behavior for an ant encountering a superheated leaf or branch surface is to move to a cooler location. Although many canopy ants can run at speeds approaching 10 cm s⁻¹ (Yanoviak et al. 2016), the lethal effects of high temperatures on insects increase rapidly with increasing temperature (Denlinger and Yocum 1998). Thus, slower ants foraging on large branches risk overheating, and this explains why the ants observed in this study avoided temperatures well below their CT_{max}. Under natural circumstances, canopy ants commonly establish foraging trails lateral to the upper surfaces of fully exposed branches. However, this is a potentially problematic trade-off because the reticulate structure of canopy substrates already limits foraging to preferred pathways shared by multiple potentially competing species (Clay et al. 2010; Yanoviak et al. 2012, 2016). The results of this study show that the risk of overheating further constrains microhabitat use by ant workers in an environment that is akin to deserts (Hood and Tschinkel 1990).

Finally, we did not measure the surface temperatures of liana (woody vine) stems in the canopy for this project, but the higher water content of lianas versus tree branches may buffer their surfaces against thermal extremes. Lianas provide various resources for ants (Yanoviak and Schnitzer 2013; Yanoviak 2015) and appear to be preferentially used by foraging ants (Clay et al. 2010); whether lianas also provide more thermally favorable pathways remains to be determined. Regardless, thermal extremes clearly are among the many risks faced by wingless cursorial insects like ants foraging in the forest canopy (e.g., Yanoviak et al. 2005).

Conclusions

Tropical ectotherms are particularly vulnerable to temperature extremes (Janzen 1967; Diamond et al. 2012; Sinervo et al. 2010). The results of this study provide field-based insight into the behavioral and morphological adaptations that allow ants to navigate the heterogeneous thermal landscape of the tropical forest canopy. We found clear behavioral avoidance of experimentally superheated surfaces as well as species-specific recovery responses to heating. These behavioral adaptations, and the variation in responses among ant species, could be clues into how this important ecological group will fare in future climate

scenarios (e.g., Stuble et al. 2013). Ultimately, this study reinforces the importance of measuring microenvironmental conditions at scales appropriate to the organism (Potter et al. 2013).

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Author contribution statement SPY, AYS, and BJA conceived and designed the experiments. MES, BJA, and AYS performed the experiments. SPY, MES, BJA, RK, and AYS analyzed the data. MK provided editorial advice, and all authors contributed to writing the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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