

Extreme Insolation: Climatic Variation Shapes the Evolution of Thermal Tolerance at Multiple Scales

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ABSTRACT: The climatic variability hypothesis (CVH) is a cornerstone of thermal ecology, predicting the evolution of wider organismal thermal tolerance ranges in more thermally variable environments. Thermal tolerance ranges depend on both upper and lower tolerance limits (critical thermal maxima [CT_{max}] and critical thermal minima [CT_{min}]), which may show different responses to environmental gradients. To delineate the relative effects of mean and extreme temperatures on thermal tolerances, we conducted a within-latitude comparative test of CVH predictions for army ants (Dorylinae) at multiple scales: across elevations, in seasonal versus aseasonal forests, and in subterranean versus surface microhabitats. Consistent with the CVH, thermally buffered subterranean species had narrower thermal tolerance ranges. Both CT_{min} and CT_{max} decreased with elevation for subterranean species. In contrast, aboveground species (those exposed to insolation) showed a decrease in CT_{min} but no change in CT_{max} across elevations. Furthermore, greater seasonal temperature variation in dry forests correlated with increased CT_{max} but not CT_{min} . These patterns suggest that CT_{max} and CT_{min} respond to different abiotic selective forces: habitat-specific exposure to extreme insolation corresponds to CT_{max} differences but not to CT_{min} variation. We predict that increasingly frequent heat spikes associated with climate change will have habitat-specific physiological consequences for ectothermic animals. Models predicting climate change impacts should account for species microhabitat uses and within-latitude differences in temperature seasonality.

Keywords: altitude, microclimate, macrophysiology, maximum critical temperature, minimum critical temperature.

Introduction

The climatic variability hypothesis (CVH) has made profound impacts on the fields of evolutionary physiology and spatial ecology (Janzen 1967; Addo-Bediako et al. 2000; Ghalambor et al. 2006; Gaston et al. 2009). CVH models predict the evolution of species with wide thermal tolerance ranges in environments with variable temperatures as well as the evolution of thermal specialists with narrow tolerance ranges in thermally stable environments (Janzen 1967). Macroscale comparative tests of this hypothesis have focused on latitudinal variation in thermal physiology, showing general decreases in species' thermal tolerance breadth toward the equator (Deutsch et al. 2008; Sunday et al. 2011). However, latitude gradients vary in mean temperature and in temperature seasonality, and thermal adaptations are selected for by both types of pressures (Payne and Smith 2017). Within-latitude comparisons among sites and microhabitats with different climatic ranges can provide powerful alternatives to latitudinal comparisons for testing hypotheses about adaptive variation in thermal physiology. Tropical sites at the same latitude can vary dramatically in mean annual temperature across elevations, while elevationally matched sites in tropical rain shadows versus on windward sides of mountains can have similar mean temperatures but different temperature seasonality (MacArthur 1972).

As small-bodied and abundant poikilotherms, ants are tractable models for studying physiological adaptation on scales from global (Diamond et al. 2012b; Diamond 2017) to microclimatic (McGlynn et al. 2010; Kaspari et al. 2015; Bujan and Kaspari 2017). Here we use a within-latitude geographic sample of army ants (Formicidae: Dorylinae) to test for CVH-predicted associations of thermal tolerance with differences in mean temperature and temperature variation on several scales. We took advantage of army ants' broad distribution across precipitation gradients, elevational ranges, and their diversity of subterranean to surface-active behavior (O'Donnell and Kumar 2006; Longino and Colwell 2011;

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O'Donnell et al. 2011; Bulova et al. 2016) to investigate relative effects of local geographic and microclimate variation on the evolution of thermal tolerance. Within sites, subterranean versus aboveground active army ant species experience similar mean temperatures but different temperature variation on diel timescales (Parton and Logan 1981; Baudier et al. 2015; Baudier 2017). Species living in dry forests also experience greater diel and seasonal temperature variation than species and populations in wet forests (Janzen 1986; Forsyth and Miyata 2011).

Because upper thermal tolerances (CT_{max}) and lower thermal tolerances (CT_{min}) can vary independently (Hoffmann et al. 2013; Bishop et al. 2016), we assayed both measures of thermal tolerance. We used standard dynamic thermal ramping assays (Lutterschmidt and Hutchison 1997; Diamond et al. 2012a; Oberg et al. 2012) to estimate CT_{max} and CT_{min} for individual worker ants. We tested whether variation in CT_{max} , CT_{min} , or thermal tolerance breadth ($CT_{max} - CT_{min}$) matched the predictions of CVHs on three different geographic scales, while accounting for body size effects (fig. 1).

Elevational Predictions

Adiabatic Cooling. Adiabatic cooling causes directional change in mean annual temperature across elevations but does not affect temperature variation (MacArthur 1972; Lazaridis 2011). On the basis of only adiabatic cooling, the CVH therefore predicts that high-elevation species will have both lower CT_{max} and CT_{min} but that thermal tolerance breadth will not covary with elevation. Previous tests of the relationship between thermal tolerance and elevation have yielded mixed results, many of which suggest less elevational change in CT_{max} than CT_{min} (Brattstrom 1968; Gaston and Chown 1999; Karl et al. 2008; Bishop et al. 2016; Oyen et al. 2016).

Extreme Insolation. Net temperature changes across elevation clines are not exclusively adiabatic. On clear days, solar radiation (particularly ultraviolet) is more intense at high elevations than at low-elevation sites (Leigh 1975; Blumthaler et al. 1997). Surface-foraging army ants at high elevations may encounter rare but extreme surface heating

Climatic Variability Hypothesis

More variable environments select for wider thermal tolerance breadth but changes in mean temperature do not

	Surface species			Subterranean species		
	CT_{max}	CT_{min}	Breadth	CT_{max}	CT_{min}	Breadth
Elevation	-	-	No change	-	-	No change
Seasonality	+	-	+	+	-	+

Extreme Insolation hypothesis

Climatic Variability Hypothesis + disproportionate effect of extreme heat on CT_{max}

	Surface species			Subterranean species		
	CT_{max}	CT_{min}	Breadth	CT_{max}	CT_{min}	Breadth
Elevation	No change	-	+	-	-	-
Seasonality	+	No change	+	+	No change	+

Weak link hypothesis

greater selective pressure on small caste thermal tolerance in variable environments

	Surface species			Subterranean species		
	CT_{max}	CT_{min}	Breadth	CT_{max}	CT_{min}	Breadth
Size	+(small)	-(small)	+	+(large)	-(large)	+

Figure 1: Predictions of three hypotheses related to the biogeography of thermal tolerance in social insects. Plus sign denotes a predicted positive relationship, minus sign denotes a predicted negative relationship, no change denotes a prediction of no relationship; shaded boxes denote predictions supported by the results of this study; boxes outlined in bold show differences between extreme insolation hypothesis predictions and the climatic variability hypothesis alone.

as a result of insolation in open areas (or via sun flecks) when skies clear (Kumar and O'Donnell 2009). If extreme insolation is an important selective force, CT_{max} may not decline and could even rise in high-elevation organisms. However, CT_{min} likely responds to nighttime low temperatures and was not predicted to be affected by extreme insolation but rather was predicted to correspond solely to adiabatic patterns in mean temperature across elevation gradients.

Soil Microhabitat Predictions

Diel Variation Buffering. In tropical forests, diel thermal variation is often greater than seasonal (MacArthur 1972), and subsoil habitats are buffered from daily high (daytime) and low (nocturnal) temperature fluctuations (Parton and Logan 1981; Baudier et al. 2015; Baudier and O'Donnell 2016). Aboveground army ant species are exposed to greater substrate insolation and more variable air temperatures. The CVH predicts that aboveground species will have both higher CT_{max} (Baudier et al. 2015) and lower CT_{min} than sympatric belowground species.

Extreme Insolation. In keeping with the extreme insolation hypothesis, we predicted the effect of microhabitat on CT_{max} to be greater at high elevations because of rare but extreme surface heating events. Diel fluctuations in air temperatures are buffered underground, so high CT_{max} values relative to mean ambient temperature should be seen only in aboveground active species at high elevations.

Tropical Seasonality Predictions

Rain shadow effects cause greater diel, seasonal, and elevational thermal variability in tropical dry forests as compared with tropical wet forests, much of which is driven by variation in insolation.

Annual-Scale Variation. Tropical dry forests experience greater seasonal variation in cloud cover and canopy cover than aseasonally wet forests, which results in seasonally superinsolated forest floors in tropical dry forests (Janzen 1986; Forsyth and Miyata 2011).

Diel-Scale Variation. Seasonal periods of dryness and clear skies in tropical deciduous dry forests cause increased diel variation in air and ground temperature because of higher daytime insolation and higher nighttime thermal reradiation as compared with evergreen rainforests (Gates 2012).

Elevational Variation. Adiabatic cooling associated with elevation change is reduced for wet slopes (lapse rate $\approx 6^\circ\text{C}/\text{km}$) as opposed to dry slopes (lapse rate $\approx 10^\circ\text{C}/\text{km}$) because of

heat generated by condensation (MacArthur 1972). We sampled sites in Northern Costa Rica decreasing in seasonality from west to east (figs. 2B, A1; figs. A1, A2 are available online). We predicted that thermal tolerance breadth would increase with tropical seasonality from eastern Atlantic sites to western Pacific sites, particularly as a result of change in CT_{max} , because extreme high temperatures are likely closer to upper thermal tolerance limits of species in these regions than low temperature extremes are to minimum thermal limits (Sunday et al. 2011).

Other Moderating Factors

In our analyses we accounted for two potentially important covariates of thermal tolerance: ant worker body size (Ribeiro et al. 2012) and phylogeny (Felsenstein 1985; Diamond et al. 2012b).

Body Size. Thermal tolerance range often increases with body size within ant species because larger workers often have higher CT_{max} and lower CT_{min} than smaller nestmates (Ribeiro et al. 2012; Wendt and Verble-Pearson 2016), but interspecific body size effects on thermal tolerance vary widely among ant subfamilies (Oberg et al. 2012). To account for both within-species and between-species effects of body size, we included body size (head capsule width) of all subject ants as a covariate in analyses. Because small social insect workers are often more thermally sensitive, we predicted that a disproportionate amount of adaptive change in thermal tolerance across ecological clines would be due to small castes (Baudier and O'Donnell 2017). Worker ant physiology can be selected upon simultaneously by opposing forces favoring morphological specialization (such as task specialization), and the need for nestmates to work together in the same thermal environment (Baudier and O'Donnell 2017). Army ants have size-polymorphic workers that group forage, with castes of different sizes cooperating under a range of thermal conditions.

Phylogeny. Moderate to high levels of surface activity have evolved multiple times in Neotropical doryline army ants (Brady 2003; Brady et al. 2014; Bulova et al. 2016; Winston et al. 2016; Borowiec 2017; fig. A2). We tested for the effect of species relatedness on the relationship between species soil microhabitat use and thermal tolerance by using Felsenstein's method for independent contrasts (Felsenstein 1985).

Methods

Field Sites and Sampling

Army ants were collected from six sites in northern Costa Rica that varied in elevation and climatic seasonality, from the Pacific slope of the continental divide to the relatively

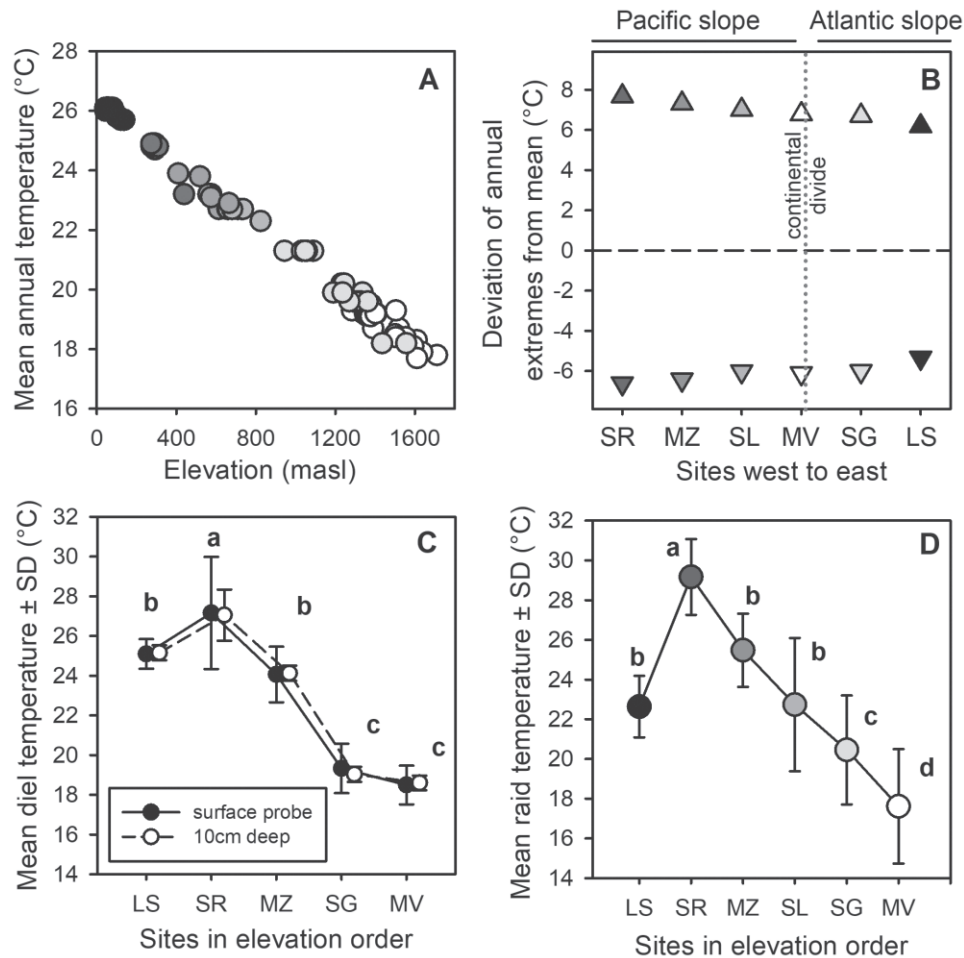


Figure 2: Overview of study site climate data. *A*, Tight relationship between elevation and Bioclim 1960–1990 estimates for mean annual temperature. *B*, Rain shadow effects on seasonal temperature variability. Upward-pointing arrows are Bioclim 1960–1990 estimates of maximum temperature warmest month minus mean temperature; downward-pointing arrows are Bioclim estimates of mean annual temperature minus minimum temperature coolest month. *C*, Mean and variance of temperatures as measured by iButtons placed on the forest floor versus at tuna oil baits 10 cm underground. Letters denote significant differences among site means. *D*, Mean and standard deviation of surface raid temperature at each site. Letters denote significant differences among site means.

aseasonal Atlantic slope (figs. 2, A1). Pacific slope sites included lowland tropical dry forest and tropical moist forest in Santa Rosa National Park (10°53'N, 85°46'W), sampled May 3–31, 2016; transitional premontane moist forest near Maritza Biological Station (10°58'N, 85°30'W), sampled May 16–18, 2016; and premontane moist forest in the San Luis Biological Reserve (10°15'N, 84°50'W), sampled July 31, 2014, and August 3, 2014 (Janzen 1986; Nadkarni and Wheelwright 2000). Atlantic slope sites consisted of lowland tropical rain forest near La Selva Biological Station (10°25'N, 84°01'W), sampled June 17–July 5, 2014, and premontane rain forests near San Gerardo Biological Station (10°21'N, 84°47'W), sampled March 22–24, 2015, and April 21–24, 2016 (McDade 1994; Nadkarni and Wheelwright 2000). Premontane and montane moist and

wet forests on or near the continental divide were sampled in the area of Monteverde (10°18'N, 84°49'W) July 8–August 4, 2014, and April 6–May 1, 2016 (Nadkarni and Wheelwright 2000).

Within each site, daily trail walking was used to locate surface-foraging army ant species, while subterranean foraging species were baited using tuna oil in covered bait pits (7 cm diameter × 10 cm depth; Meisel 2006; O'Donnell et al. 2007; Kumar and O'Donnell 2009; O'Donnell et al. 2011; Baudier et al. 2015). Transects consisted of five baits spaced 5 m apart and >3 m away from trails within forested areas. When possible, army ant raids (group-foraging ants) were followed back to their temporary nests (bivouacs), and bivouacs were tracked over time to delineate colonies within each site. In cases where bivouacs were not located, en-

counters of conspecific raids >300 m apart within 24 h were assumed to be different colonies, because this exceeds the longest recorded raid distance for army ant colonies (Rettenmeyer 1963).

Sampling for thermal tolerances consisted of $N = 3,988$ worker ants from $N = 128$ colonies of $N = 15$ army ant species (fig. 1). Once collected, ants were transported to the laboratory while they were provided access to water in excess (via moist paper towel), followed by thermal tolerance assays beginning within 3 h of collection. Coordinates and elevation at the site of each army ant colony's raid were recorded using handheld global positioning system units (Garmin GPSMAP 62S, Garmin, Olathe, KS).

Species Soil Microhabitat Use

Fifteen Neotropical army ant species were used in this study. An index of surface activity (ISA) was calculated for each species by z -transforming and averaging the literature-based index of army ant surface activity (IL) formulated by Bulova et al. (2016) and the relative eye size index (RESI; small worker proportion of eye height to head width at antennal insertion) of Baudier et al. (2015) as follows, where σ is the standard deviation across species:

$$ISA = \frac{[(IL - \overline{IL})/\sigma_{IL}] + [(RESI - \overline{RESI})/\sigma_{RESI}]}{2}.$$

This method characterizes microhabitat use for species for which behavior is not well studied and reduces the effect of large eye facet bias among nocturnal species (Moser et al. 2004), such as *Neivamyrmex gibbatus* and *Eciton mexicanum*. ISA is used as a proxy for species differences in soil microhabitat for all analyses.

Thermal Tolerance Assays

Thermal tolerance assays were conducted using the standard dynamic method of thermal ramping for both CT_{max} and CT_{min} (Lutterschmidt and Hutchison 1997; Oberg et al. 2012; Bishop et al. 2016). Ants were placed individually in 1.5-mL conical microcentrifuge tubes stoppered with cotton. From 14 to 20 ants from each colony were run together in either CT_{max} or CT_{min} assays. A dry heat block (Thermal-Lok 1 and Thermal-Lok 2, USA Scientific, Orlando, FL) was used for CT_{max} assays, and a Tropicooler (Benchtop Hot/Cold Block Incubator, Boekel Scientific, Feasterville, PA) was used for CT_{min} assays. CT_{min} blocks were precooled to 15°C, while CT_{max} blocks were preheated to 30°C at the start of each assay. Ants were exposed to incrementally more extreme temperatures at a rate of 1°C every 10 minutes. At the end of each 10-min interval, subjects were checked for movement in response to light tapping 10 s after removal from blocks. CT_{max} was considered 1°C less than the temperature

at which an individual lost mobility response in heat tolerance assays. CT_{min} was defined as 1°C greater than the temperature at which an individual lost mobility response in cooling assays. Thermal tolerance breadth was calculated as the colony mean CT_{max} – colony mean CT_{min} .

Five ants per colony ranging in body size were placed in microcentrifuge tubes stoppered with cotton but were kept aside to test for possible unexpected sources of mortality, such as illness or inadvertent injury during transport. Two colonies with more than two-fifths control ant mortality before the end of each thermal tolerance assay were not included in this study. Ninety-nine percent of other controls survived for the duration of each assay.

Body Size Measurements

Each subject ant was photographed with a dissecting-scope mounted digital camera (Amscope). Individual head width at antennal insertion was measured using ImageJ version 1.46 software and was used as an index of body size in all analyses. Relative eye height to head width at antennal insertion was also measured in this manner among the five smallest caste workers within each species. This RESI was used to calculate ISA for each species.

Ambient Temperature Data Collection

We analyzed three types of climate data: empirical data from temperature loggers placed at tuna baits, empirical infrared temperatures at surface raids, and extrapolated bioclimatic estimates of temperature annual range.

Soil and Surface Temperatures during Raids. At the site of each tuna trap, two pairs of thermochron iButton data loggers (Maxim Integrated, San Jose, CA) were deployed, recording temperature at the soil surface and 10 cm below the surface every 5 min for three consecutive days. Surface iButtons were shielded from direct sunlight both by plastic housings and by a thin white plastic plate that covered the traps to reduce error from direct insolation to the metal body of the probe. Because baits were in forested areas, ephemeral sunflecks through the canopy were the major source of ground-warming insolation measured by these probes. These probe temperatures were used to compare diel variation in temperature above- and belowground across sites (except for the San Luis reserve). A subset of 10-cm-depth iButton data 1 h before and after subterranean ants were observed at baits was used to estimate subterranean raid temperatures.

Surface Temperatures during Raids. Ground surface temperatures at aboveground army ant raids were recorded across elevations and sites using handheld infrared thermometers (BAFX Products, Milwaukee). Consecutive in-

frared surface temperature measurements were taken 25 cm apart along the portion of the column and raid front that was exposed to the surface.

Bioclim Climate Data. Temperature annual range (BIO₇) and mean annual temperature (BIO₁) data for 1960–1990 were extracted from WorldClim bioclimatic variables (BIOCLIM) by using coordinates of army ant raids via the geographic coordinate system WGS1984 at a resolution of 1 km² (Hijmans et al. 2005, 2008). Elevation was tightly correlated with Bioclim-estimated mean annual temperature across sampled sites (fig. 2A), justifying the use of elevation as a proxy for mean annual temperature in our analyses. Bioclim estimated temperature annual range (BIO₇) was used in all analyses as a proxy for thermal seasonality.

Statistical Analyses

All analyses were performed in R version 3.1.2 (R Development Core Team 2014).

Testing Climatic and Physiological Predictors of Thermal Tolerance. To analyze effects of body size and local climate on thermal tolerance differences among army ant species, linear mixed models were fitted (using lmer in package lme4) with either CT_{min} or CT_{max} as a response variable (Bates et al. 2015). Fixed predictors were ISA (microhabitat use), head width (body size), elevation, annual range in temperature, surface activity × head width interactions, surface activity × elevation interactions, and surface activity × annual temperature range interactions; random variables were colony identification (ID), site (Santa Rosa, San Gerardo, San Luis, Monteverde, Maritza, or La Selva), and taxon (species or subspecies, as depicted in fig. A2). Nonsignificant predictor variables were eliminated until the minimum model was reached. Significance of variables was estimated by likelihood ratio tests, assessed by χ^2 statistics. Akaike information criterion (AIC) for each variable informed order of comparisons, but the *P* value estimated by likelihood ratio tests alone was used to qualify significance of each variable. Separate analyses were conducted for CT_{max} and CT_{min} data. The effects of the same predictor variables on mean colony thermal tolerance breadth (mean CT_{max} – mean CT_{min}) were analyzed in a separate analysis that did not account for random effects of colony. The equations from the results of these analyses are reported in the appendix, available online. Thermal tolerance, colony, and species identifications as well as collection data of all subjects sampled are available from the Dryad Digital Repository: <https://dx.doi.org/10.5061/dryad.kr4031f> (Baudier et al. 2018).

Accounting for Phylogeny. New World army ants are monophyletic, with a well-supported genus-level phylogeny (Brady

2003; Brady et al. 2014; Borowiec 2017) and species-level resolution within *Labidus* and *Eciton* (Barth et al. 2015; Winston et al. 2016; fig. A2). Pagel's λ was calculated for the main predictor and response variables to test for phylogenetic constraints and to determine the need for phylogenetic correction ("Methods for Calculating Pagel's λ "; Freckleton et al. 2002). Phylogenetically independent contrasts were calculated from army ant species mean values for ISA, CT_{max}, CT_{min}, and thermal tolerance breadth (Felsenstein 1985), using the ape package in R (Paradis et al. 2004). Branch lengths were set to 1, with the exception of a polytomy in genus *Neivamyrmex*, for which branch lengths between species were set to 0. ISA was regressed against each of these three predictor variables. Significance and slopes were compared between phylogenetically corrected and non-corrected species level regressions.

Testing for Local Ambient Temperature Differences. To test for the effects of site and soil depth on ambient temperature, iButton temperature means and ranges across all sampled days were set as response variables in separate linear mixed model analyses, which accounted for transect and bait ID as random variables and site and soil depth (0 vs. 10 cm) as fixed predictors. Predictor variables were eliminated until the minimum required model was reached (Quinn and Keough 2002). AIC values for each predictor variable informed order of comparison and elimination. Infrared recorded temperatures of surface raids were analyzed in a separate similar mixed model analysis, which included site and species as fixed predictor variables and raid ID as a random variable.

Results

Environmental Temperatures

Mean ambient temperature differed significantly across our sample sites, as measured by iButtons ($\chi^2 = 103.68$, *df* = 4, *P* < .001; fig. 2C) and ground surface temperatures at surface raids ($\chi^2 = 128.89$, *df* = 5, *P* < .001; fig. 2D). Recorded temperatures generally declined with elevation (fig. 2A, 2C, 2D). Surface raid temperatures did not differ among species within sites ($\chi^2 = 14.35$, *df* = 12, *P* = .279). Mean temperatures were not significantly different at soil surface and 10 cm belowground within sites ($\chi^2 = 0.02$, *df* = 1, *P* = .900), but temperatures 10 cm below soil surface were on average 4.6°C less variable than surface temperatures within each site because of soil buffering of diel variation ($\chi^2 = 25.49$, *df* = 1, *P* < .001; fig. 2C). Diel temperature range differed among sites ($\chi^2 = 18.40$, *df* = 4, *P* = .001). Daily temperature range was 7.3°C greater in Santa Rosa than in La Selva (*t* = 2.11, *z* = 3.44, *P* = .005), and daily range was 6.5°C greater in Santa Rosa than in Monteverde (*t* = 1.60, *z* = 4.04, *P* < .001).

Bioclim mean annual temperatures were tightly correlated with elevation (fig. 2A), so we used elevation as a proxy for mean annual temperature in subsequent analyses. Bioclim estimated temperature annual range (maximum temperature hottest month – minimum temperature coldest month) increased from wet forest to dry forest sites (fig. 2B).

Elevation and Soil Microhabitat

CT_{min} decreased as elevation increased ($\chi^2 = 8.62$, $df = 1$, $P = .003$; fig. 3C). Surface-active species had lower CT_{min} than subterranean species within each elevation, and the slope of the elevational effect on CT_{min} did not vary with species' ISA ($\chi^2 = 2.39$, $df = 1$, $P = .122$; fig. 3C). In contrast, the effect of elevation on CT_{max} differed by ISA: subterranean ants decreased in CT_{max} as elevation increased, while surface-active species' CT_{max} remained relatively stable across elevations ($\chi^2 = 4.86$, $df = 1$, $P = .027$; fig. 3A). Supporting the CVH, colony mean thermal tolerance breadth increased with ISA, but the magnitude of this effect was greater at high elevations than in the lowlands; only aboveground species increased in thermal tolerance breadth at high elevations, while breadth actually decreased with elevation in subterranean species ($\chi^2 = 5.69$, $df = 1$, $P = .017$; fig. 3E) because of a steeper decrease in CT_{max} than CT_{min} with elevation for belowground species.

Soil Microhabitat and Body Size

Aboveground species were on average larger than belowground species, and, as expected, colony thermal tolerance breadth increased as mean colony head width increased ($\chi^2 = 5.85$, $df = 1$, $P = .016$; fig. 4A). However, most species' size ranges overlapped with other species, justifying interspecific comparisons of microhabitat effects on the relationship between body size and thermal tolerance. CT_{max} increased with body size among individual workers, but this body size effect was stronger for subterranean species ($\chi^2 = 57.52$, $df = 1$, $P < .001$; fig. 4B–4D). CT_{min} decreased as body size increased, but this effect was also stronger for subterranean species ($\chi^2 = 40.15$, $df = 1$, $P < .001$; fig. 4).

Seasonality

Consistent with the extreme insolation hypothesis, CT_{max} was significantly higher in environments with greater thermal seasonality (temperature annual range) independent of mean temperature ($\chi^2 = 12.35$, $df = 1$, $P < .001$; fig. 3B), while annual temperature range was not a significant predictor of CT_{min} ($\chi^2 = 3.17$, $df = 1$, $P = .075$; fig. 3D). Colony mean thermal tolerance breadth was not significantly different across the seasonality gradient ($\chi^2 < 0.01$, $df = 1$, $P = .963$; fig. 3F).

Phylogenetic Effects

Pagel's λ estimates for species mean CT_{min} and thermal tolerance breadth showed no evidence for significant phylogenetic constraint: the estimated value of λ was not significantly different from 0 (λ values of 0 indicate no constraint). For CT_{max} and ISA, there was evidence for weak to moderate phylogenetic constraint; Pagel's λ values below 0.43 (for CT_{max}) and 0.22 (for ISA) were significantly unlikely. Because there was some evidence for phylogenetic effects on some of our main predictor and response variables, we accounted for phylogenetic effects in our correlation analyses by performing phylogenetically independent contrasts (PICs).

PICs suggested that the effect of surface activity on species mean thermal tolerance breadth and CT_{max} was similar with and without correcting for species relatedness (fig. 5). Thermal tolerance breadth increased with degree of aboveground activity using both raw data ($F_{1,13} = 16.05$, $R^2 = 0.52$, $P = .001$; $y = 31.50 + 2.61x$; slope SE = 0.60) and independent contrasts ($F_{1,12} = 5.76$, $R^2 = 0.27$, $P = .034$; $y = -0.46 + 2.46x$; slope SE = 1.02). CT_{max} also increased with surface activity in both uncorrected data ($F_{1,13} = 18.97$, $R^2 = 0.56$, $P = .001$; $y = 37.84 + 1.74x$; slope SE = 0.40) and when accounting for relatedness ($F_{1,12} = 8.42$, $R^2 = 0.36$, $P = .013$; $y = -0.34 + 1.72x$; slope SE = 0.59). The negative relationship between CT_{min} and surface activity was weakly significant using uncorrected data ($F_{1,13} = 6.05$, $R^2 = 0.27$, $P = .029$; $y = 6.29 - 0.83x$), but this relationship was nonsignificant when accounting for relatedness ($F_{1,12} = 2.21$, $R^2 = 0.09$, $P = .163$).

Discussion

A key prediction of the CVH is adaptation to local thermal conditions for resident species (Janzen 1967; Ghalambor et al. 2006); both upper and lower thermal tolerances should evolve in response to ambient thermal conditions. Tests of the CVH often use mean temperatures as indicators of local thermal conditions and ignore microclimatic effects on variation in species thermal environments. Here we present the first phylogenetically controlled empirical test of the CVH that incorporates temperature variation and microhabitat effects along a latitude-matched tropical seasonality gradient.

As predicted by the CVH, we found that thermal tolerance varied with elevation; adiabatic cooling apparently selected for variation in thermal tolerances among Neotropical army ant species. However, consistent with the extreme insolation hypothesis, our data also suggested there were pervasive effects of high-temperature extremes as drivers of thermal tolerances, particularly at the upper thermal tolerance limits (CT_{max}). As outlined below, several patterns in

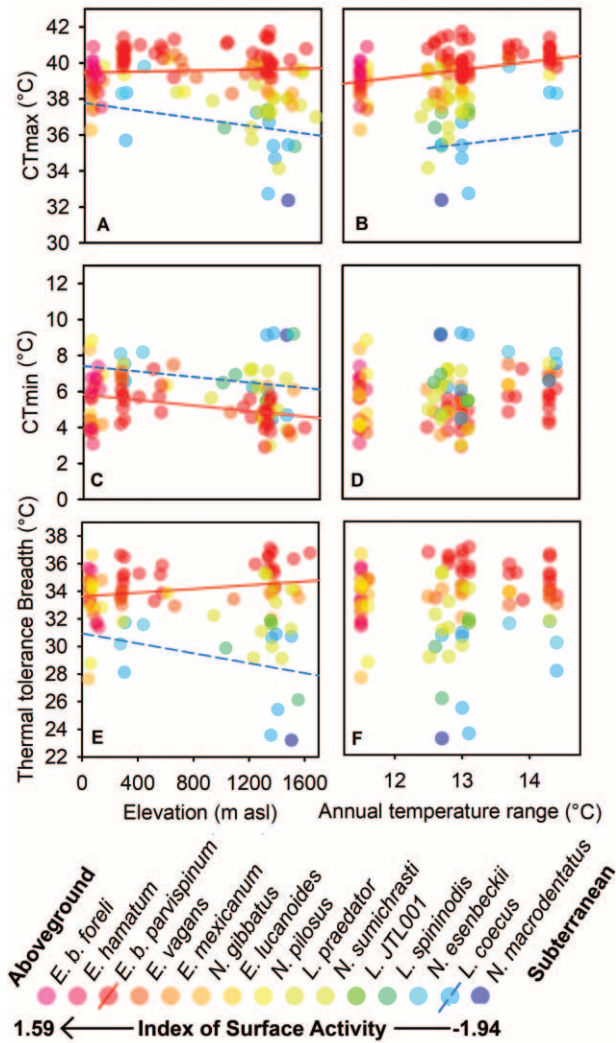


Figure 3: Effect of elevation (m asl), annual temperature range (Bioclim), and species soil microhabitat use on CT_{max} , CT_{min} , and thermal tolerance breadth ($CT_{max} - CT_{min}$). Circles are colony means, and lines represent significant trends among the most widely sampled surface-active species *Eciton burchellii parvispinum* (red line) and the most widely sampled subterranean species *Labidus coecus* (blue line).

our data set indicate that thermal variability and extreme/rare temperature events are important modifiers of thermal adaptation. These patterns were evident when examining thermal tolerance variation in relation to site seasonality (rain shadow effects), microhabitat (ISA), and body size. This pattern of temperature variability predicting CT_{max} is consistent with the results of a global meta-analysis of large-bodied ectotherms (Clusella-Trullas et al. 2011), suggesting that extreme high-temperature effects are widespread. Our findings also add to the widening body of evi-

dence suggesting that rare extreme temperature events have a high selective pressure on species upper thermal tolerances (Clusella-Trullas et al. 2011; Smale and Wernberg 2013; Kingsolver and Buckley 2017). Though extreme pulse events in the environment are inherently difficult to study, their selective pressure is strong, and extreme events should be considered as relevant environmental factors in the evolution of physiology (Grant et al. 2017).

Insolation and Asymmetry in CT_{max} versus CT_{min}

CT_{min} has been widely held as more climate responsive than CT_{max} when using local mean temperatures as a predictor variable (Sunday et al. 2011; Bishop et al. 2016). In contrast, we demonstrated stronger patterns in CT_{max} than CT_{min} when accounting for temperature variability across microhabitat and tropical seasonality gradients. Unlike minimum environmental temperatures, maximum environmental temperatures encountered by small tropical ectotherms are often in the form of highly insolated and superheated surfaces and boundary layers (Cerdá 2001; Kaspari et al. 2015). Extreme but relatively rare temperatures that disproportionately affect CT_{max} are not well represented by mean annual temperatures, potentially masking correlations between temperature variability and CT_{max} in previous studies. There is increasing evidence that rare, extreme thermal events play important roles in selecting for thermal capacities (Smale and Wernberg 2013; Dowd et al. 2015; Kingsolver and Buckley 2017). We propose the extreme insolation hypothesis—that CT_{max} is disproportionately driven by high-temperature extremes, often in the form of direct or indirect insolation—as an extension and refinement of the CVH.

Soil Microhabitat

Our findings on the microhabitat scale supported the CVH, showing increased thermal tolerance breadth among surface-active species that encounter greater diel variation in temperature. Soil microhabitat use was a significant predictor of both CT_{max} and CT_{min} , but a stronger relationship was observed in CT_{max} . This fit the prediction that CT_{max} is more heavily selected upon by rare but extreme temperatures, while CT_{min} is more heavily selected upon by local mean temperatures.

Elevation and Soil Microhabitat

Within sites, belowground species had narrower thermal tolerance ranges than aboveground species, supporting CVH predictions. However, the relationship between thermal tolerance and elevation was complex. Although both subterranean and aboveground species decreased in CT_{min} toward

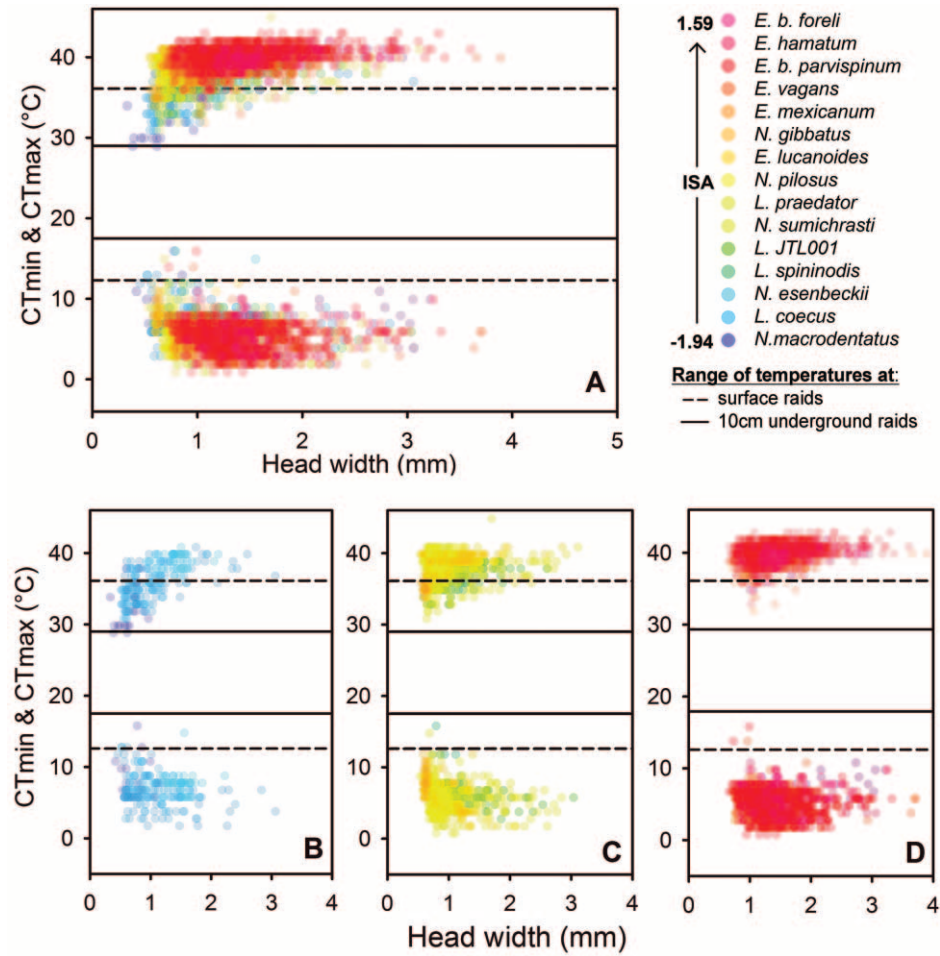


Figure 4: A, Effect of body size on individual thermal tolerance, with aboveground species overlaid atop belowground species ($N = 3,998$ ants). Species are listed in order of increasing index of surface activity (ISA); warmer colors are more surface-active species, and cooler colors are more subterranean species. Solid lines are maximum and minimum underground raid temperatures, and dashed lines are maximum and minimum surface raid temperatures. B, Effect of body size on individual thermal tolerance for *Neivamyrmex macrodentatus*, *Labidus coecus*, and *Nomamyrmex esenbeckii*. C, Effect of body size on individual thermal tolerance for *Labidus spininodis*, *Labidus JTL001*, *Labidus praedator*, *Neivamyrmex pilosus*, *Eciton lucanoides*, and *Neivamyrmex gibbatus*. D, Effect of body size on individual thermal tolerance in *Eciton mexicanum*, *Eciton vagans*, *Eciton burchellii parvispinum*, *Eciton hamatum*, and *Eciton burchellii foreli*.

high elevations, the CT_{max} response to elevation was dependent on species microhabitat use. Subterranean species decreased in CT_{max} , while surface-active species' CT_{max} was relatively stable across elevations. This pattern is consistent with extreme insolation hypothesis predictions, assuming that aboveground species are selected upon more heavily by rare but extreme temperatures (Clusella-Trullas et al. 2011; Kingsolver and Buckley 2017). We show that microhabitat differences modify the relationship between thermal tolerance and elevation, offering a possible explanation for inconsistent relationships between CT_{max} and elevation in other studies (Gaston and Chown 1999; Karl et al. 2008; Angilletta 2009). The magnitude of elevational change in CT_{min} and CT_{max} for subterranean species was consistent

with estimated slopes of CT_{min} change with elevational in other studies on ants (Bishop et al. 2016).

Body Size and Soil Microhabitat

Consistent with previous studies (Ribeiro et al. 2012; Baudier et al. 2015; Wendt and Verble-Pearson 2016), body size was a significant predictor of thermal tolerance, with smaller conspecifics having narrower thermal tolerance breadths than larger nestmates. However, the magnitude of the body size effect on thermal tolerance range was diminished in surface-dwelling species. This supports the hypothesis that in social insect colonies, temperature selection operates most strongly on the most thermally sen-

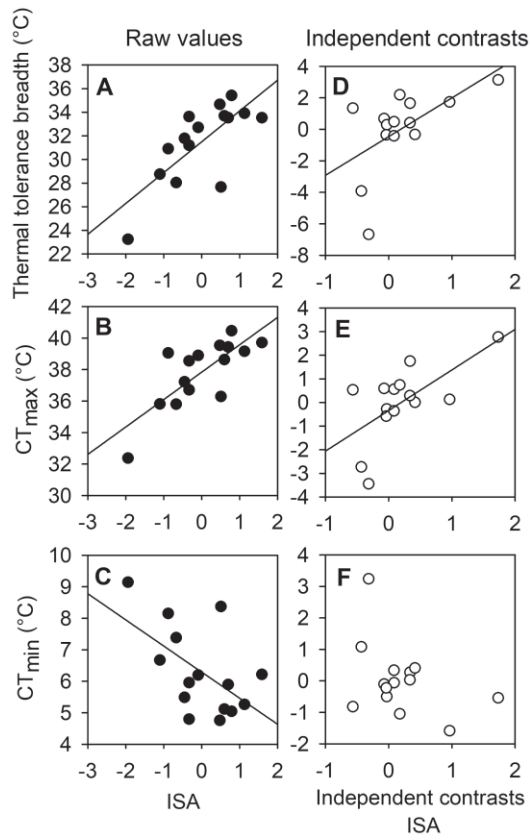


Figure 5: Correlation between species index of surface activity (ISA) and species means for three thermal tolerance metrics. Filled circles represent raw data, and open circles represent phylogenetic independent contrasts that control for relatedness.

sitive (in this case, small) nestmates, in other words, that small workers show the greatest evolutionary responses to ambient thermal conditions (Baudier and O'Donnell 2017). This weak-link hypothesis accurately predicted the patterns in body size versus thermal tolerance that we observed from belowground to forest floor; this is also consistent with other studies that report lack of body size effects in canopy species that experience greater diel temperature variation than we report here (Kaspari et al. 2015). Broadly, this suggests that mean species thermal tolerance estimates could misrepresent small-caste associated climate change costs for social species, particularly for species inhabiting historically thermally stable environments. In army ants specifically, increasing ambient thermal variation may have disproportional impacts on army ant colony fitness via decreases in small caste performance (Baudier and O'Donnell 2017).

Seasonality

CT_{max} increased with annual temperature range in surface and subterranean species. However, only CT_{max} (and not

CT_{min} or thermal tolerance breadth) increased in seasonal versus aseasonal forests. This pattern suggests that species inhabiting lowland wet forests may be especially susceptible to directional increase in temperature associated with climate change. Our finding that low variability habitats select for narrow thermal tolerance ranges is consistent with macrophysiological studies (Sunday et al. 2011), but our finding that variation in CT_{max} (rather than CT_{min}) is the major driver of this pattern across a tropical seasonality gradient (caused by a rain shadow) is novel.

Caveats

The critical thermal limit data presented here characterize the physiological limits of mobility across temperature clines, but other important biological processes such as growth, digestion, and reproduction may have distinct thermal constraints (Angilletta 2009). Thermal performance breadths for these physiologically demanding activities are likely narrower than critical thermal limits and merit comparison to the patterns presented in this study. Although we found no evidence of adult acclimation improving thermal tolerance assay performance in these tropical species (“Test of Acclimation Effects in *Eciton burchellii parvispinum*”), we did not explore the extent to which rearing temperatures may play an important role in setting adult thermal tolerance. Regardless of mechanisms at play in setting these critical limits, the ubiquity of high CT_{max} in high-variance environments across many spatial scales suggests that this end result phenotype is adaptive.

Conclusions

In this tropical within-latitude study, we found general support for the CVH, but fit to the data was improved by incorporating extreme insolation effects on surface temperature into predictions. Our findings suggest that the interpretation of elevation patterns in lieu of microclimate and seasonality data can mask important aspects of thermal tolerance trends. This is especially the case for CT_{max} , calling into question the previously held notion that CT_{max} is less responsive to ecological gradients than CT_{min} (Brett 1956; Addo-Bediako et al. 2000; Gaston et al. 2009; Sunday et al. 2011; Diamond and Chick 2017; fig. 1). Our results suggest that CT_{max} is highly responsive to local environmental temperature fluctuations and that high temperature extremes are particularly relevant. Rare high-temperature events can drive differentiation in heat tolerance across sympatric species that occupy different soil microhabitats, particularly at high elevation. By incorporating a sympatric microhabitat thermal variability gradient into a large-scale biogeographic study, we have tested and shown support for the extreme insolation hypothesis, suggesting that CT_{min} is responsive to

ambient mean temperature, while CT_{max} may be strongly selected upon by rare extreme heat events.

We tested the extreme insolation hypothesis from scales of a few centimeters to hundreds of kilometers, but our results have implications for larger macrophysiological clines. Our findings support the notion that increasing incidence of extreme heat spikes associated with climate change will have strong consequences for small-bodied ectotherms. Such heat spikes can alter complex interspecies interactions on an ecological scale (Denny et al. 2009; Diamond et al. 2016) and may result in either extirpation or evolutionary changes in thermal physiology of organisms inhabiting these changing environments (Grant 2017; Grant et al. 2017).

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Intermediately surface-active army ants of species *Eciton vagans* follow one another in a surface column raid for several meters before going into a hole underground in Santa Rosa National Park. Photo credit: Kaitlin M. Baudier.