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Rain shadow effects predict population differences in thermal tolerance of leaf-cutting ant workers (*Atta cephalotes*)

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Abstract

Tests of hypotheses for the evolution of thermal physiology often rely on mean temperatures, but mounting evidence suggests geographic variation in temperature extremes is also an important predictor of species' thermal tolerances. Although the tropics are less thermally variable than higher latitude regions, rain shadows on the leeward sides of mountains can experience greater diel and seasonal variation in temperature than windward sites. Rain shadows provide opportunities to test predictions about the relationships of extreme temperatures with thermal physiology while controlling for latitude. We tested the hypothesis that populations of leaf-cutting ants (*Atta cephalotes*) in leeward, montane, and windward sites in Costa Rica would differ in upper thermal tolerances (CT_{max}) of workers. As predicted from rain shadow effects via extreme high temperatures, the leeward rain shadow site yielded the highest mean CT_{max} (rain shadow site 42.1 ± 0.3°C, Montane site 38.2 ± 0.5°C, and windward site 38.2 ± 0.3°C). This suggests that high-temperature extremes in tropical rain shadow forests can select for higher thermal tolerances. CT_{max} increased with worker body size within sites, but CT_{max} increased with body size more gradually at the two lowland sites, as predicted if local high temperatures selected more strongly on the most thermally vulnerable society members (small workers). This suggests that warmer lowland climates selected for colonies with less variation in heat tolerance than cooler high elevation climates.

Abstract in Spanish is available with online material.

KEYWORDS
altitude, caste, Costa Rica, global warming, maximum critical temperature, weak link hypothesis

1 | INTRODUCTION

Rare but extreme environmental events can have a disproportionately large influence on the evolutionary trajectory of organismal physiology (Denny, Hunt, Miller, & Harley, 2009; Grant et al., 2017). Temperature extremes are particularly relevant to the study of thermal tolerance evolution across geographic thermal gradients (Clusella-Trullas, Blackburn, & Chown, 2011). According to the thermal adaptation hypothesis, also referred to as Janzen's rule or the climatic variability hypothesis, species living in more thermally variable environments are expected to have wider thermal tolerance breadths (Janzen, 1967). Consistent with this hypothesis, tropical species are often more thermally specialized relative to high-latitude species (Ghalambor, Huey, Martin, Tewksbury, & Wang, 2006; Sunday, Bates, & Dulvy, 2011). While minimum thermal tolerance (CT_{min}) generally correlates with geographic temperature clines driven by elevation and latitude, the relationship between maximum thermal tolerance (CT_{max}) and local climate is often relatively

weak (Deutsch et al., 2008; Diamond & Chick, 2017; Gaston & Chown, 1999; Karl, Janowitz, & Fischer, 2008; Sunday et al., 2011) or non-existent (Addo-Bediako, Chown, & Gaston, 2000; Huey et al., 2009; Kimura, 2004). Based on these large-scale trends, recent reviews suggested that CT_{max} is less selected upon by climate, or may be more phylogenetically constrained, than CT_{min} (Diamond & Chick, 2017; Sunday et al., 2019). However, this generalization is at odds with mounting evidence that CT_{max} is strongly related to species-typical thermal microclimates within sites (Baudier, Mudd, Erickson, & O'Donnell, 2015; Kaspari, Clay, Lucas, Yanoviak, & Kay, 2015), perhaps even more so than CT_{min} (Baudier, D'Amelio, Malhotra, O'Connor, & O'Donnell, 2018). The two most commonly assessed macrophysiological temperature clines (latitude and elevation) represent confounded gradients in mean temperature and in temperature variability. Therefore, studies that distinguish the relative evolutionary importance of mean temperature versus variation in temperature are needed (Payne & Smith, 2017). This motivates the search for alternative local environmental predictors of CT_{max} variation. Here, we study CT_{max} variation across a tropical rain shadow gradient as a geographic model to advance current understanding of the evolution of thermal tolerances.

1.1 | Tropical rain shadows as a tool for studying local adaptations to mean temperature versus temperature variability

Tropical forests generally experience less change in temperature across seasons of the year than do temperate regions (MacArthur, 1972; Sunday et al., 2011). However, tropical mountain ranges can generate rain shadows that cause within-latitude contrasts in precipitation and in temperature variability (Galewsky, 2009). For example, the windward side of the continental divide in the Caribbean lowlands of Costa Rica is characterized by relatively warm but stable temperatures across the diel cycle (day vs. night) and across seasons (rainy vs. dry seasons) (Figure 1; Baudier et al., 2018). Contrastingly, on the leeward side of the Costa Rican continental divide lie lowland seasonal dry forests, which experience pronounced rain shadow effects, particularly when the trade winds are strong during the dry season (Janzen, 1986). The higher aridity of climate and increased seasonality of rainfall in tropical rain shadows causes greater diel and seasonal variation in temperature than in elevation-matched windward forests. Rain shadows generate some of the most extreme high and low temperatures experienced at tropical sites (Forsyth & Miyata, 2011; Gates, 2012; Janzen, 1986; MacArthur, 1972). These pronounced differences in leeward versus windward climates of mountains are seldom accounted for in studies of how thermal tolerance relates to climate. We suggest tropical rain shadows provide novel opportunities for analyzing adaptation to mean and/or extreme temperatures on an overlooked geographic scale. Elevation-matched and latitude-matched sites across rain shadow gradients can have similar mean annual temperatures but differ in maximum temperatures and temperature range.
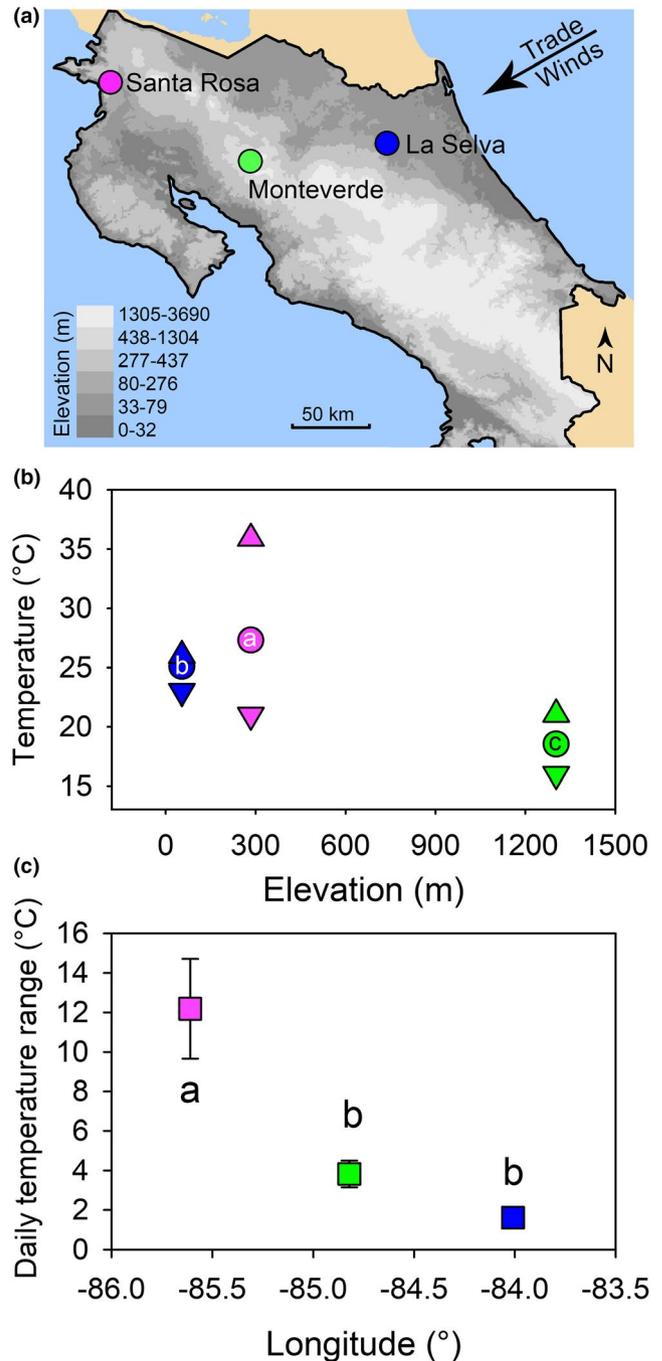
FIGURE 1 Climate and geological details of the three sites sampled in this study: low elevation Atlantic rain forests of La Selva, Premontane wet forests of Monteverde near the continental divide, and seasonal Pacific dry forests of Santa Rosa. (a) Topographic map depicting three field sites. (b) Average daily mean (●), maximum (▲), and minimum (▼) temperatures at each of the elevationally diverse sites. (c) Rain shadow effect on daily temperature range in northwestern Costa Rica. Squares are mean daily temperature range, error bars are 95% confidence intervals. See Table S1 for further information regarding analyses comparing site temperature

Cooler higher elevation sites in the middle of this seasonality cline allow for additional tests of whether maximum thermal tolerance (CT_{max}) is selected upon by temperature means versus temperature variation.

1.2 | The weak link hypothesis: patterns of individual CT$_{max}$ variation in social species

Insects are small-bodied poikilothermic ectotherms, and so are often used as subjects in macrophysiological analyses of geographic variation in thermal physiology (Deutsch et al., 2008; Diamond & Chick, 2017; Diamond, Sorger, et al., 2012; Gaston et al., 2009). As social insects, ants provide additional opportunities to explore individual differences in thermal tolerance. Within eusocial insect colonies, subclasses of workers, such as size-castes, can differ in thermal physiology (Baudier & O'Donnell, 2018; Ribeiro, Camacho, & Navas, 2012). When this is the case, basing an assessment of a species' thermal tolerance can mask negative impacts on colony fitness via differential effects on the most thermally vulnerable colony members (Baudier & O'Donnell, 2017). Because there may be especially strong selection on the most thermally vulnerable subclasses, such as small-bodied workers, the weak link hypothesis predicts that within-group body size-associated variation in CT$_{max}$ will be lower in social insect species or populations that are adapted to environments with more extreme temperatures (Baudier et al., 2018, 2015; Baudier & O'Donnell, 2017). We tested this prediction by measuring how worker sub-caste body sizes related to thermal tolerance in different climates generated by rain shadow effects and elevation.

We used the leaf-cutting ant *Atta cephalotes* as a model species to study geographic and climatic variation in thermal tolerance (CT$_{max}$). *Atta cephalotes* is abundant across elevations and on both sides of the continental divide in northern Costa Rica (Freeman & Chaves-Campos, 2016; Perfecto & Vandermeer, 1993; Rockwood, 1973) and has size-differentiated worker sub-castes (Burd, 2000). We measured worker CT$_{max}$ across a gradient in both diel and seasonal temperature extremes generated by a tropical rain shadow, and across a gradient in mean temperatures generated by elevation (see Figure 1 for relationship between sites and mean vs. extreme temperatures). We compared population differences in upper thermal tolerance in latitude-matched windward (aseasonal lowland rainforest), leeward (seasonal lowland dry forest), and continental divide (high elevation) sites. Based on site differences in mean temperatures and in temperature extremes, we made two main predictions. First, if mean temperature differences drive the evolution of CT$_{max}$, we expected *A. cephalotes* workers from the cooler high elevation site to have lower CT$_{max}$ than workers from either of the warmer lowland sites (Table 1). As a corollary, we used the weak links hypothesis to predict that smaller (vulnerable) workers would be more robust to high temperatures at both of the warmer lowland sites because high temperatures would select most strongly on CT$_{max}$ of the smallest (weak link) workers; therefore, we predicted a greater effect of body size on CT$_{max}$ at our high elevation site (Table 1). Second, if occasional extreme high temperatures are a major selective force on CT$_{max}$, we predicted that mean CT$_{max}$ would be higher at the lowland rain shadow site than at either of the other two sites.

TABLE 1 Predicted relationships of geography with mean colony CT$_{max}$ and with the slope of CT$_{max}$ versus worker body size for leaf-cutter ants

Geographic parameter	Mean CT$_{max}$	Slope of CT$_{max}$ versus Size
Elevation (low: warmer vs. high: cooler)	SR & LS > MV	SR & LS < MV*
Rain Shadow (in: extreme temperatures vs. out: moderate temperatures)	SR > MV & LS*	SR < MV & LS

Note: "*" indicates the patterns that had significant statistical support from our findings. Whether or not the site was in a rain shadow was a better predictor of mean CT$_{max}$, but elevation was a better predictor of the slope between CT$_{max}$ and body size due to weak link effects. Abbreviations: LS, La Selva (lowland and not in rain shadow); MV, Monteverde (high elevation and not in rain shadow); SR, Santa Rosa (lowland and rain shadow).

2 | METHODS

2.1 | Ant collections

We studied thermal tolerance of the leaf-cutting ant *A. cephalotes* across its elevational range extremes in Costa Rica, sampling high elevations near the continental divide and lowlands on both the Pacific rain shadow (leeward) and Caribbean less variable (windward) slopes of the Tilarán mountain range (Figure 1). All fieldwork was performed during the rainy seasons of 2014 and 2016 in premontane wet forests of Monteverde (N10.31°, W84.31° 1296–1311 m asl), lowland rainforests of La Selva Biological Station (N10.42°, W84.02° 50–58 m asl), and seasonal dry forests of Santa Sosa National Park (N10.85°, W85.64° 297–300 m asl). Although we searched the continental divide near Monteverde up to over 1,800 m above sea level (m asl), *A. cephalotes* was only common at and below circa 1,300 m asl, suggesting that our sampling was representative of the elevational extremes for this species in the region. Within each site, active and mature nests of *A. cephalotes* at least 500 m linear distance apart from one another were selected. Twenty worker ants across the range of body sizes we encountered were collected from the tops of the nest mounds and transported (within 2 hr of collection) to the laboratory to use as subjects in thermal tolerance assays. Three colonies were sampled in Santa Rosa and La Selva, and two colonies were sampled in Monteverde. Voucher specimens from each colony were deposited in the entomology collection of the Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA.

2.2 | Ambient temperature measurements

Daily maximum, minimum, and mean temperatures were calculated from temperatures recorded round-the-clock in each site using iButton thermochron data loggers (Maxim Integrated). Temperature sampling was concurrent with CT$_{max}$ sampling of the ants (rainy seasons of 2014 and 2016). Each temperature logger was placed in a plastic housing and beneath a plastic sheet (to shield the probe from direct insolation)

and inserted beneath the leaf litter on the forest floor. Loggers recorded surface soil temperature once every 5 min for three continuous days. Loggers were arranged in transects of three, spaced 5 m apart from one another and 3 m into the forest alongside dirt trails at each site. A total of eight such transects were deployed in Santa Rosa, nine in Monteverde, and two in La Selva. We also obtained bioclim extrapolations of local temperatures at each site (Fick & Hijmans, 2017).

2.3 | Thermal tolerance assays

We ran standard dynamic heat tolerance assays (Diamond, Nichols, et al., 2012; Ribeiro et al., 2012), using digital dry heat blocks (USA Scientific). Ants were placed in 1.5 ml microcentrifuge tubes stoppered with cotton at the top to prevent access to thermal refuge in the tubes during the assay. Tubes were placed in the dry heat block and started at 3°C, increasing in temperature at a rate of 1°C every 10 min. Every 10 min, we checked for loss of mobility response to light tapping on the tube. For each ant, lack of motor response to tapping was interpreted as having surpassed the critical thermal maximum (CT_{max}). For each colony, five ants across the range of caste sizes were kept in tubes but not put into heat blocks for the duration of the assays, to ensure that factors other than increasing temperature did not account for loss of mobility. None of the control ants in this study lost mobility, suggesting that the cause of loss of mobility was due to temperatures experienced within the heat blocks rather than handling and confinement.

2.4 | Body size

After performing CT_{max} assays, we collected all ant workers and measured body sizes from head capsules. Each ant was photographed at 15–40× magnification (depending on size of ant) using a scope-mounted digital camera. ImageJ software was used to measure the width of heads at eye-height from photographs (Schneider, Rasband, & Eliceiri, 2012). A micrometer accurate to 0.1 mm was used to convert head width measurements from pixels to millimeters. Head width was used as a proxy for body size across all analyses.

2.5 | Calculations and analyses

All analyses were conducted in R version 3.4.4 (R Core Team, 2018). Linear mixed-effects models were used to test for differences in daily temperature maximum, minimum, range, and mean across sites using the lmer function in the package lme4 (Bates, Mächler, Bolker, & Walker, 2015). We fitted a mixed-effect model with daily temperature range as a response variable, site as a fixed factor, and transect and probe as nested random factors, then tested for significance of the fixed factor (site) using a type II Wald chi-square analysis. The same procedure was used to test for differences in daily temperature maximum, minimum, and mean. Post hoc Tukey HSD tests were used for pairwise comparisons of each climatic metric among sites.

To test whether the slope of the CT_{max} versus body size relationship differed significantly across sites, we used log-log transformed data. Following this transformation, no sites showed a curvilinear relationship between size and CT_{max}, enabling us to use a mixed-model ANCOVA. We first fit a linear mixed-effect model (using lmer) with colony ID as a random factor, log-transformed CT_{max} as a response variable and site (three levels: La Selva, Monteverde, Santa Rosa), body size (log-transformed head width), and the interaction between size and site as fixed factors. We tested for significant differences in the slopes and intercepts of the three trends between body size and CT_{max} (weak link effects) among the three study sites via a type II Wald chi-squared test using the ANOVA function in the "car" package (Fox et al., 2012). We conducted pairwise post hoc comparisons among the sites for mean CT_{max} and slope of CT_{max} with body size using least squares estimations (functions lsmeans and lstrends, respectively).

3 | RESULTS

3.1 | Geographic temperature variation

Daily temperature range, daily maximum temperature, daily minimum temperature, and daily temperature mean all differed across study sites (Figure 1; Table S1). The leeward (rain shadow) site Santa Rosa had both the greatest diel range in temperature and the greatest daily maximum temperatures. However, windward and leeward lowland sites (La Selva and Santa Rosa) did not differ in nightly minimum temperature. This was consistent with bioclim extrapolations of diel temperature range across sites (Fick & Hijmans, 2017). Mean daily temperature differed significantly across all sites, with the leeward rain shadow lowland site of Santa Rosa being hottest despite being slightly higher in elevation than the lowland windward site of La Selva. Mean daily temperature in both lowland sites was also significantly higher than in the high elevation site of Monteverde.

3.2 | Mean CT_{max}

Mean ± *SE* CT_{max} for the three study sites were as follows: Santa Rosa 42.1 ± 0.3°C, Monteverde 38.2 ± 0.5°C, and La Selva 38.2 ± 0.3°C. Sites differed significantly in mean CT_{max} (log-transformed data; X^2 = 26.15, *df* = 2, *p* < .001) and CT_{max} corresponded more closely to rain shadow effect predictions than to elevation (see predictions in Table 1; results in Table 2): post hoc analyses revealed that mean CT_{max} in Santa Rosa was significantly higher than La Selva or Monteverde, but that Monteverde and La Selva did not differ in Mean CT_{max} (Table 2).

3.3 | Size versus CT_{max} relationship (weak links)

The relationship between ant size and CT_{max} differed significantly across sites; in this case, the strongest site differences corresponded to elevation differences rather than to rain shadow

TABLE 2 Analysis of deviance table for type II Wald chi-square test performed for the linear mixed-effects model: $LogCT_{max} \sim Site * LogHW + (1 | Colony)$. Log transformation of CT_{max} ($LogCT_{max}$) and head width (LogHW) was done to normalize distributions

Type II Wald chi-square			
Fixed factors	X^2	*df*	*p*
Site	26.1	2	<0.001*
LogHW	91.1	1	<0.001*
Site: LogHW	13.8	2	0.001*

Pairwise site comparisons of mean CT_{max}					
Contrasts	**Estimate**	**SE**	**df**	**t-ratio**	**p**
Monteverde—La Selva	0.002	0.011	4.87	0.182	0.982
Santa Rosa—La Selva	−0.045	0.010	4.88	−4.438	0.016*
Santa Rosa—Monteverde	−0.047	0.011	4.87	−4.154	0.021*

Slopes of log(CT_{max}) versus log(head width) across sites						
Site	**Slope**	**SE**	**df**	**Low 95% CL**	**Upper 95% CL**	**Group**
La Selva	0.092	0.023	152	0.048	0.137	B
Monteverde	0.178	0.021	150	0.137	0.219	A
Santa Rosa	0.076	0.021	152	0.035	0.118	B

Note: Pairwise comparisons of mean CT_{max} across sites were estimated by least square means using the lsmeans function in r. Comparisons of the slope in CT_{max} across head widths were performed across sites using the lstrends function. Groupings in slope comparisons were based on 95% confidence intervals.

*Significance ($\alpha = 0.05$).

effects (log-transformed data; $X^2 = 13.78$, *df* = 2, *p* = .001; Figure 2; Tables 1 and 2). In cooler high elevation Monteverde, CT_{max} declined significantly more rapidly with decreasing body size than in either low elevation site, where the heat tolerance of small-bodied workers was closer to the CT_{max} of large workers. Large workers were about equally thermally robust across all sites, with much of the slope difference among sites being driven by differences in small worker CT_{max}.

4 | DISCUSSION

Here, we report that populations of the same species differ in mean heat tolerance (CT_{max}) across a rain shadow gradient, suggesting that tropical dry forest climates on the leeward side of mountains selected for higher CT_{max}. Elevational differences in mean temperatures were not associated with mean differences in CT_{max}; however, geographic variation in leaf-cutter ant worker CT_{max} was dependent on interactions with worker body size. Elevation-related site differences in CT_{max} were strongest for the smallest workers, which are the most thermally vulnerable colony members. This pattern is consistent with predictions of the weak link hypothesis. Extreme temperatures were a better predictor of mean CT_{max}, while mean environmental temperature was a better predictor of the slope of

size versus CT_{max} (Table 1). This is the first demonstration of these patterns across populations of the same widely distributed species. Similar patterns were observed in a comparative study across multiple species in Neotropical army ants (Baudier et al., 2018).

In this study, we used ambient temperature data collected from the forest floor, and foragers from the forest floor to estimate thermal tolerances. It is important to note that some subset of leaf-cutting ant workers also forage in tree canopies, which are typically more thermally variable than the forest floor (Kaspari et al., 2015). Also, we conducted this study in the rainy season, but dry season daytime high temperatures in Santa Rosa are of even greater magnitude. The estimates of warming tolerances presented here should therefore be considered conservative. Furthermore, canopy-active species can also face compounded threats of desiccation (Hood & Tschinkel, 1990).

The pattern of decreased effects of body size on worker tolerance variation in warmer lowland sites supports the weak link hypothesis, which predicts that selective pressure against the least tolerant nestmates will be amplified when ambient maximum temperatures are near the least tolerant colony member's CT_{max} (Baudier & O'Donnell, 2017). Such was the case in this study for the warmer lowland sites (whether they were or were not within a rain shadow) relative to a cooler high elevation site. Large workers were about equally thermally robust across all sites, with much of
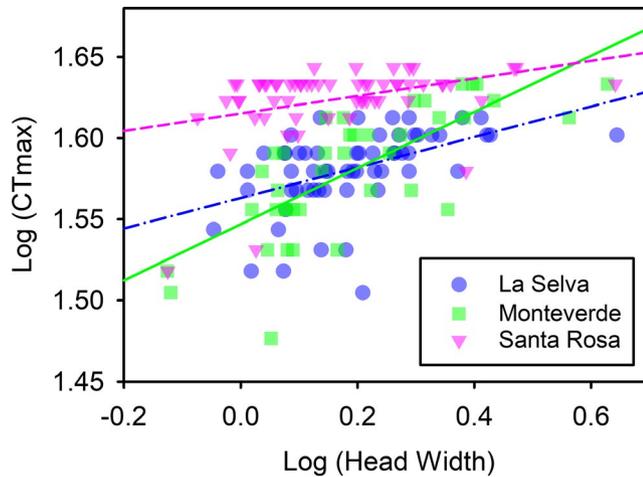
FIGURE 2 Relationship between body size and heat tolerance (CT_{max}) varying across sites sampled: low elevation Atlantic rain forests of La Selva, Premontane wet forests of Monteverde near the continental divide, and seasonal Pacific dry forests of Santa Rosa. Trends depicted are result of a mixed model ANCOVA performed using log-log normalized data for CT_{max} (°C) and Head Width (mm). Further detail regarding untransformed relationships can be found in supplementary information. Darker points represent overlapping data

the slope difference between high and low elevation sites being driven by changes in small worker CT_{max}. We did not explore the underlying mechanistic factors involved in setting these striking phenotypic differences in CT_{max}, which could include rearing temperature, adult acclimation, or fixed genetic differences. However, the patterns are consistent with theory that suggests thermal variation is a selective pressure on individual and colony-level thermal physiology.

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DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.k0p2ngf4b (Baudier & O'Donnell, 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.