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Thermal adaptation and phosphorus shape thermal performance in an assemblage of rainforest ants

MICHAEL KASPARI,[1,2,6] NATALIE A. CLAY,[1,2] JANE LUCAS,[1,2] SHAI REVZEN,[5] ADAM KAY,[3] AND STEPHEN P. YANOVIAK[4]

[1]*Graduate Program in Ecology and Evolution, Department of Biology, University of Oklahoma, Norman, OK 73019, USA*
[2]*Smithsonian Tropical Research Institute, Balboa, Republic of Panama*
[3]*Department of Biology, University of St. Thomas, St. Paul, MN 55105, USA*
[4]*Department of Biology, University of Louisville, Louisville, KY 40292, USA*
[5]*Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI 48109, USA*

Abstract. We studied the Thermal Performance Curves (TPCs) of 87 species of rainforest ants and found support for both the Thermal Adaptation and Phosphorus-Tolerance hypotheses. TPCs relate a fitness proxy (here, worker speed) to environmental temperature. Thermal Adaptation posits that thermal generalists (ants with flatter, broader TPCs) are favored in the hotter, more variable tropical canopy compared to the cooler, less variable litter below. As predicted, species nesting in the forest canopy 1) had running speeds less sensitive to temperature; 2) ran over a greater range of temperatures; and 3) ran at lower maximum speeds. Tradeoffs between tolerance and maximum performance are often invoked for constraining the evolution of thermal generalists. There was no evidence that ant species traded off thermal tolerance for maximum speed, however. Phosphorus-Tolerance is a second mechanism for generating ectotherms able to tolerate thermal extremes. It posits that ants active at high temperatures invest in P-rich machinery to buffer their metabolism against thermal extremes. Phosphorus content in ant tissue varied three-fold, and as predicted, temperature sensitivity was lower and thermal range was higher in P-rich species. Combined, we show how the vertical distribution of hot and variable vs. cooler and stable microclimates in a single forest contribute to a diversity of TPCs and suggest that a widely varying P stoichiometry among these ants may drive some of these differences.

Key words: ants; boundary layer; community; ectotherms; functional traits; phosphorus; thermal adaptation; thermal tolerance; tradeoffs; tropical forest; vertical stratification.

INTRODUCTION

Thermal Performance Curves (TPCs, Fig. 1) capture the temperature dependence of behaviors that serve as proxies for fitness; they are a basic tool for predicting population, community, and ecosystem responses to a warming environment (Huey and Slatkin 1976, Tracy and Christian 1986, Diamond et al. 2012, Sunday et al. 2012). Yet we still lack a deep understanding of how and why the TPCs of species vary in time and space, or how and why TPCs vary among the species in a given ecosystem (Huey and Kingsolver 1993, Chown et al. 2002, Angilletta 2009). Here, we addressed these questions with an assemblage of 87 Panama ant species (Kaspari et al. 2015) exploring how and why the run speed of worker ants—a correlate of the rate at which they do the colony's work—varies with temperature.

Thermal Adaptation is the main theory underlying TPCs (Janzen 1967, Levins 1968, Lynch and Gabriel 1987, Deutsch et al. 2008, Huey et al. 2009, Sunday et al. 2011). It predicts two adaptive responses to an ectotherm's thermal environment (Fig. 1). First, a population's maximum thermal limit, CT_{max}, should track the maximum temperature of its environment, T_{env}. Second, the range of temperatures at which a population is active will track the range of temperatures the population experiences: variable environments should favor thermal generalists.

Testing Thermal Adaptation: when are hotter environments more variable?

Most of the strongest tests of Thermal Adaptation have compared populations from different locations along the latitudinal gradient, as tropical populations tend to experience both warmer, and less seasonally variable environments (Janzen 1967). Higher latitude populations of a variety of ectotherms tend to have broader thermal tolerances (Deutsch et al. 2008, Sunday et al. 2011); the heat tolerances of *Drosophila* populations tracked environmental temperature (but only in arid environments, Kellermann et al. 2012); and ectotherms from cold environments tend to have lower maximum growth rates (Frazier et al. 2006). However, decreased maximum performance in species from colder and more seasonally

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[6]E-mail: mkaspari@ou.edu

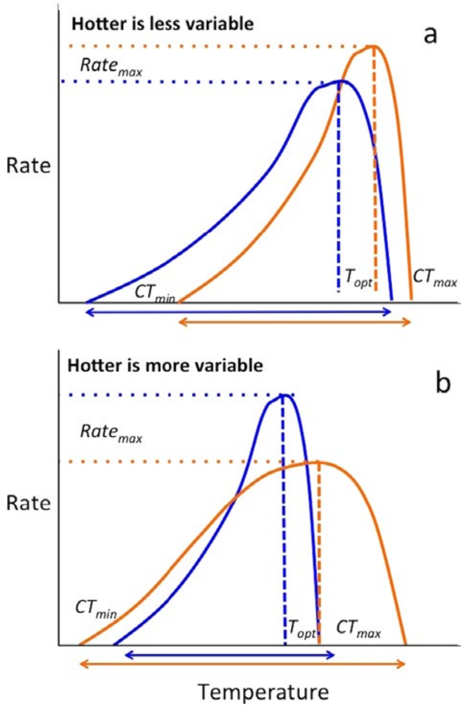

FIG. 1. Predicted shapes of Thermal Performance Curves (TPCs) under Thermal Adaptation when temperature mean and variance are positively or negatively correlated. Horizontal arrows represent the range of environmental temperature experienced by individuals in a pair of habitats, orange and blue. (a) Where warmer environments are less variable (e.g., along the latitudinal gradient), warmer environments generate temperature specialists with higher maximum performance. (b) Where warmer environments are more variable, warmer environments favor temperature generalists with lower maximum performance. CT_{min} and CT_{max} are the low and high temperatures at which performance ceases, $Rate_{max}$ is the peak performance rate, and T_{opt} is the temperature at which $Rate_{max}$ occurs.

variable environments, while consistent with Thermal Adaptation's "jack-of-all-trades" prediction, can't rule out one alternative: that it is the colder environment alone that drives the pattern (Frazier et al. 2006, Kingsolver and Huey 2008).

To cut this Gordian knot requires thermal gradients where the mean and variance of temperature are negatively correlated. Earth's biotas are often distributed vertically, with some shaded from the sun's rays and others experiencing the full range of daily solar radiation. Consider Earth's forests. In a Panamanian forest, we found that boundary layers in the forest canopy generate surfaces averaging 10°C warmer than temperature maxima in the litter 30 m below and, since temperatures cooled and equilibrated in the absence of solar radiation, canopy temperatures were also more variable (Kaspari et al. 2015). Forests canopies thus allow a strong test of Thermal Adaptation's prediction of a performance tradeoff that can be distinguished from the simpler working hypothesis of "Hotter is Better" (Frazier et al. 2006, Kingsolver and Huey 2008). Specifically, in the forest canopy, Thermal Adaptation predicts lower peak performance while Hotter is Better predicts higher peak performance. Thermal adaptation also predicts broader TPCs for species living in the canopy.

Mechanisms underlying TPCs

The mechanisms for Thermal Adaptation, not surprisingly, have often been grounded in tradeoffs between generalists and specialists (Levins 1968, Rosenzweig 1995). In this view, thermal generalists are "jacks-of-all-trades" and pay a performance penalty compared to populations in the more stable environment (Angilletta 2009). Tradeoffs assume that populations shed the costly metabolic machinery that is required for life in temperatures they don't experience. Instead, they accumulate machinery for life in the environments they experience most frequently (Lynch and Gabriel 1987, Hochachka and Somero 2002). Despite the guiding role tradeoffs play in studies of trait evolution (Levins 1968, Rosenzweig 1995), there have been few convincing tests that tradeoffs constrain the shapes of TPCs (Huey and Kingsolver 1993, Angilletta 2009).

An alternative approach is to look for resources that can be directly invested by an organism toward enhancing one or more components of TPCs. Here we propose the Phosphorus-Tolerance hypothesis, which assumes that species can regulate their P-intake and shunt that P into traits that enhance CT_{max}, the temperature at which muscular control fails. In support of the first assumption, organisms foraging in different areas or on different foods can vary their ingestion of P (Walker and Syers 1976, Kaspari and Yanoviak 2008). In support of the second, we suggest two ways that P-rich tissue may promote CT_{max}. First, ribosomes are P-rich (Elser et al. 2000) and high ribosomal densities would allow the rapid upregulation of Heat Shock Proteins that prevent denaturation of other proteins under heat stress (Feder and Hofmann 1999, Rinehart et al. 2007). Second, high temperatures also carry the risk of desiccation in small insects, a risk that can be allayed by closing their spiracles (Harrison et al. 2012). Under such hypoxia, insects can still anaerobically convert P-rich arginine phosphate, via arginine kinase, to ATP. While this reaction is often associated with extreme power output (Zammit and Newsholme 1976) we suggest it may also postpone the hypoxia associated with hot, desiccating temperatures. Here, while we do not test the mechanism underlying Phosphorus-Tolerance, we do test the assumption that ants in the same community differ in their P-content, and that the P-content of an ant's tissue is positively correlated with its CT_{max}.

Testing TPC models using ant speed

Here we contrast TPCs based on quantifying worker speed in 87 ant species from the same Panama rainforest.

We measure the TPCs of workers because worker ants serve to feed and defend the colony. We measure worker speed for two reasons. First, within an ant species, worker speed, or tempo, has long been assumed to correlate with colony fitness (Oster and Wilson 1978, Sarty et al. 2006, Hurlbert et al. 2008) by enhancing the rate that ants return with food, attack, and flee from predators. There is some evidence for this assumption: when worker speeds were slowed experimentally in two species, colony food retrieval slowed along with it (Fewell 1988, Powell and Franks 2005, 2007); and when colonies of *Pheidole dentata* were experimentally imperiled by the fire ant *Solenopsis geminata*, their running speed increased (Wilson 1976). A second reason to focus on speed is its ubiquity in studies of thermal tolerance (Angilletta 2009) making our results directly comparable to a large literature on TPCs.

In a companion paper, the Thermal Adaptation hypothesis correctly predicted the higher thermal maxima, $CT_{max,}$ of canopy species (3.5°C higher for a given body size, Kaspari et al. 2015). Here we test its prediction that canopy species will show broader tolerance range ($CT_{max}-CT_{min}$), decreased peak performance activity, and decreased temperature sensitivity (i.e., smaller increases in speed with temperature) relative to understory species. We also test the Phosphorus-Tolerance Hypothesis' prediction that ants with higher tissue concentrations of P show higher CT_{max}.

MATERIALS AND METHODS

All work was conducted on Barro Colorado Island, (9°9′19″ N, 79°50′15″ W), a lowland, seasonally wet forest in Lake Gatun of the Panama Canal (Leigh 1999). BCI has a fauna of ca. 400 species of which we studied 87.

In May–July 2011, ant colonies were collected from ants nesting in the leaf litter and in the twigs and branches of the tropical canopy. We collected opportunistically, with at least one of us each day devoting 2–4 h looking for colonies and returning them to the lab for ID. Given the long tail of tropical species abundance curves, we aimed to maximize the number of species sampled at the expense of multiple colonies of the same species, with half the species represented by one colony, and the rest represented by 2–5 replicates. In each case, the curve of speed vs. temperature was the average across colonies of a species (the CV for speed at 27°C for species represented by 3, 4 and 5 samples was 53, 38, and 53, respectively). Within colonies, when speed at 27°C was measured at two different points in the ramping protocol (see below), the second speed was highly correlated with the first (second speed (cm^{-s})=0.77 (first speed)$^{0.76}$, $r^2 = 0.53$, $P < 0.0001$) though the exponent of 0.76 suggest that fastest species showed some degradation in speed the second time around.

In the lab, voucher specimens were both pinned and stored in 95% EtOH and identified to species or morphospecies. The remaining ants in the colony were placed in 15 cm diameter plastic petri dishes with cotton balls soaked in distilled water. These colonies were acclimated in a dark drawer at 27°C for 24 h.

Quantifying ant mass and whole body P

Ants from the trial, and the rest from the colony including workers, reproductives, and brood, were placed in a 1.5 ml microcentrifuge tube, which was then frozen at −80°C. Dry mass of five workers, after freeze drying, was recorded for each colony used and averaged to generate estimates of body mass.

We used a Thermo Scientific XSERIES 2 ICP-MS w/ESI PC3 Peltier cooled spray chamber and SC-FAST injection loop to estimate the whole body concentration of P (parts per million, ppm). For 26 common species of ants, a sample of 5 + workers was homogenized, diluted in nitric acid and analyzed using He/H2 collision-reaction mode.

Quantifying TPCs

We measured speed through the voluntary movement of 4–6 workers (four if equi-sized, five to six if polymorphic) in a gridded 15 × 2.5 cm petri dish above a white background and below an HD video camera. We varied temperature using a ramping protocol, moving ants from ambient temperature down to CT_{min} and then up to CT_{max} (when three species of ants were tested with a "ramping up only" protocol, CT_{maxs} were indistinguishable from conspecifics that where ramped down, then up, Kaspari et al. 2015). This measure of acute thermal tolerance, when performed over a short period of time, minimizes confounding factors of starvation or desiccation (Terblanche et al. 2011). Moreover, tests with models impaled on thermocouples showed that the largest ants reached equilibrial temperature in ca. 1 min, and the smallest in ca. 2 s (Kaspari et al. 2015). The 15–30 min duration of the cooling and heating portions of the experiment thus should have allowed ants to achieve thermal equilibrium with their surroundings.

At the outset of the experiment, ants were kept at 27°C for 15 m. Next, the surface temperature of the dish (ca. 27°C) was measured with an IR thermometer (Mastercool (T), resolution 1°C). These surface temperatures were later translated to air temperatures at the surface using thermocouples (see for details Kaspari et al. 2015). Ants were video recorded for 1 min. To cool the petri dish, a sandwich of waxed paper, 1 cm of high-density foam, and frozen cryopacs was slipped under the dish. This served to cool the dish to 20°C after ca. 15 min. When surface temperature reached 20°C, we began a second 1-min video, recording surface temperature twice more at 30 and 50s (for each video, the mean of those three temperatures is recorded as the surface temperature). To cool to CT_{min} (defined as when > half of the ants fell over and could no longer right themselves if the dish was gently shaken, or chill coma, Angilletta et al. 2007) the foam/wax paper buffer was removed, and, if necessary, a CryoPak was placed above the petri dish.

Next, the cryopacs were removed and ants were allowed to return to ambient temperature and normal behavior – grooming and running – a process that took ca. 15–30 min. Those colonies that did not recover (5 of the 154 colonies tested) were discarded from the trials. We placed the petri dish back on the white base above a commercial heating pad with a 1 cm dense foam buffer. We recorded a second 1-min video at room temperature.

We used the heating pads to warm the petri dish to a surface temperature of 35°C (ca. 15 min). We recorded ant activity at this temperature for 1 min as described herein. Finally, to achieve CT_{max} (defined as when >50% of ants exhibited loss of righting reflex, or knockdown) we allowed the Petri dish to heat to ca. 40°C, then sometimes using a second heating pad, until CT_{max} was achieved.

In sum, our protocol always cooled the ants to CT_{min} before raising them to CT_{max}.

Quantifying average ant speed

We used a video analysis system to convert the four videos for each colony trial to generate estimates of ant speed at 20, 27 (2×) and 35°C. We tracked the ants using custom image processing software written in MatLab (Mathworks 2012). We detected the ants by background subtraction of a polynomial fit to the illumination, followed by thresholding, then tracked blobs of pixels of a per-trial, manually configured area range as they moved, using a nearest neighbor matching from frame to frame. We made no attempt to resolve which ant was which when tracks intersected, since this would not change the statistics we collected. We computed the scale for the images based on the size of the dish to generate an average speed (cm^{-s}) across all the ants in a given trial-temperature.

We validated the MatLab program using 10 random videos: three each at 35°C and 20°C, 4 at 27°C. The ant tracks for the first 20 s of each video were drawn onto scale representations of the gridded petri dishes. Distance traveled by each ant was measured by tracing each track with <1 mm diameter thread, then measuring the length of thread to the nearest mm. This method had a high repeatability, when duplicated for one random ant, the second trace = 0.2 + 1.02*first trace ($r^2 = 0.998$, $n = 10$). The MatLab program precisely duplicated the trace method (MatLab = −0.02 + 1.09*Manual, $r^2 = 0.700$, $n = 10$).

Calculating 3 parameters of TPCs

For each species, we calculated three TPC parameters representing thermal sensitivity of ant speed: thermal sensitivity (the slope, or change in velocity from 20 to 35°C), the maximum average speed recorded at a given temperature for a species ($Speed_{max}$, cm^{-s}), and the thermal tolerance range (CT_{range}, $=CT_{max}-CT_{min,}$°C).

As body mass is often a strong predictor of running speed for a variety of animals (Peters 1983), including ants (Hurlbert et al. 2008)— and given that larger ants likely experienced slightly cooler temperatures above the arena surface (Kaspari et al. 2015) we used Least Square Means regression to quantify the effect of Log_{10} dry mass of ants on the three parameters. If significant, we tested the two hypotheses with the residuals of the mass regression (e.g., allowing us to compare the maximum speed for a given body size between the two habitats).

Statistics

To test the Thermal Adaptation Hypothesis, we compared the three parameters between the 25 canopy and 62 ground nesting species using a Kruskal-Wallis test. We also evaluated the assumption of tradeoffs among the parameters using Reduced Major Axis regression. We tested the Phosphorus-Tolerance Hypothesis by regressing the whole body Phosphorus content of 26 species against CT_{max}, as well as slope and $Speed_{max}$.

Phylogenetically independent contrasts (PICs) generate a conservative null model for trait evolution by assuming traits evolve through Brownian motion, that selective regimes for the clade (in this case, climate mean and variance) change at the same pace as rates of speciation; and that those speciation rates are constant (Losos 2011). PICs simulate how this evolutionary scenario reduces the independence of our species-level measurements. We conducted PICs using the *pic* function and the APE package (Paradis et al. 2004) in R (Team 2011). We relied on a genus-level phylogenetic tree (Fig. S1). We generated pruned chronograms with associated branch lengths, based on a previously published chronogram from (Moreau et al. 2006). Tip data are means for each genus. Regressions were forced through the origin (Garland et al. 1992) and carried out using the linear model function in R. We did not perform a PIC ANOVA contrasting differences in the three TPC variables between habitats, because species from four genera nested in both canopy and understory, resulting in decreased statistical power when ant genera were classified as understory, canopy, and "mixed". We did use *pic* to provide the conservative estimate of trait distribution when 1) comparing TPC variables with body mass, 2) quantifying tradeoffs among these traits, and 3) testing the Phosphorus-Tolerance hypothesis. In each case we report if patterns in the raw data persist at $P < 0.05$ using PIC.

RESULTS

We analyzed TPCs for worker ants from 25 canopy nesting species and 62 ground nesting species, sampling 8 subfamilies and 33 genera. Maximum-recorded speed

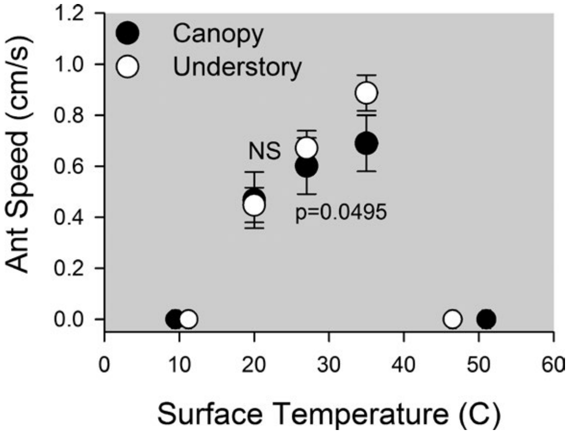

FIG. 2. Thermal performance curves of ant species nesting in the canopy and understory. (a) the LSMeans average speed (±SE) at 20, 27 and 35°C, bounded by the average CT_{min} and CT_{max} (SE's too small to be visible). *P* values reflect Kruskal-Wallis comparisons of average rates of change for speed from 20 to 27°C, and from 27 to 35°C.

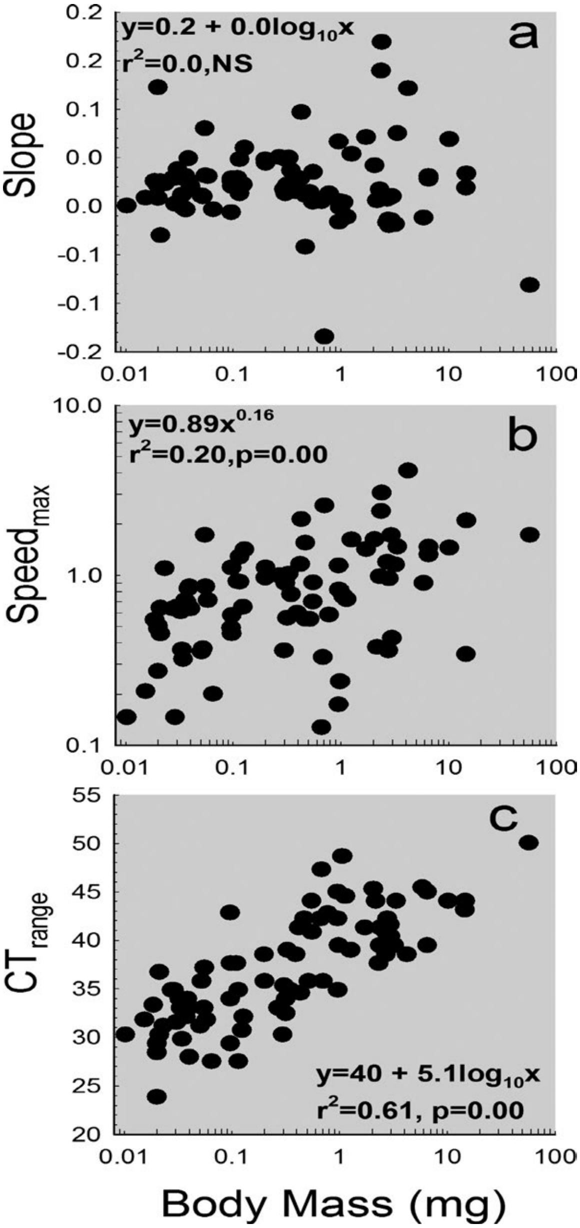

FIG. 3. Three parameters from 87 ant thermal performance curves scaled to body mass. (a) Slope represents the LSM regression estimate of the change in an ant's speed from 20 to 35°C. (b) $\text{Speed}_{\text{max}}$ (cm^{-s}) is the maximum speed recorded for the species. (c) CT_{range} (°C) is the span of surface temperatures at which ants were active.

ranged from 0.13 cm^{-s} for the canopy nesting *Camponotus* BCI_LT_19 to 4.1 cm^{-s} for the ground nesting army ant, *Eciton hamatum.* Temperature sensitivity—the slope of the TPC—ranged from −0.13 cm^{-2}/°C for *Labidus praedator* to 0.17 cm^{-2}/°C for *Leptogenys punctaticeps*. CT_{min} ranged from 4.3°C (4 species) to 18.1 °C for the ground nesting *Acropyga* sp1. CT_{max} ranged from 41°C for the ground nesting *Pheidole rugiceps* to 56°C for two species, one from each habitat. CT_{range} ($\text{CT}_{\text{max}}-\text{CT}_{\text{min}}$) ranged from 24°C for the understory *Solenopsis* ANTCNP_sp2 to 50°C for the ground nesting *Paraponera clavata*.

The mean speed increased similarly from 20 to 27°C in both habitats, (Kruskal-Wallis $X^2 = 1.6$, $P = 0.21$, Fig. 2) but more slowly from 27 to 35°C in canopy species relative to ground nesters (KW $X^2 = 3.9$ $P = 0.0495$). TPCs of canopy species thus appeared to decelerate on average relative to ground nesting species as temperatures approached 35°C.

Body size relationships

Worker mass varied from 0.01 to 57 mg and was correlated with two of three parameters we used to characterize TPCs (Fig. 3). The first is Thermal Sensitivity, which we quantified as the LSMeans slope of ant speed from 20 to 35°C (mean = 0.02, range = −0.13–0.16). Thermal Sensitivity failed to vary systematically across 3 o.m. of ant body mass (Fig. 3a, $r^2 < 0.02$). About 1 in 5 species decreased their average speed over this temperature range.

In contrast, larger ants remained active across a broader range of surface temperatures and tended to be faster (Fig 3). $\text{Speed}_{\text{max}}$, the maximum average speed of ants in the arena, varied 31-fold (mean = 0.8 cm^{-s}, range = 0.13–4.1). It increased as $\text{Mass}^{0.16}$ (Fig 3b). CT_{range}, the span of temperatures in which ants were active (mean = 37°C, range = 24–50, CV = 15) increased 5°C for every 10-fold increase in temperature; body mass accounted for 61% of the variation. CT_{max} (mean = 48°C, range = 41–56) increased as 49 + 2.8*log (body mass), ($r^2 = 0.42$, $P = 0.0001$, curve not shown). Given the importance of body size as a covariate, we size-corrected speed, CT_{max}, and CT_{range}. Going forward we used Least Squares residuals–henceforth $\text{rSpeed}_{\text{max}}$ and $\text{rCT}_{\text{range}}$–to test Thermal Adaptation and rCT_{max} to test Phosphorus Tolerance. All three body size relationships persisted at $P < 0.05$ under Phylogenetic Independent Contrasts (henceforth PIC).

Thermal adaptation

Thermal Adaptation has two key components: 1) tradeoffs in traits between temperature generalists (those with broad CT_{range}, low thermal sensitivity, and low maximum performance) and temperature specialists (those with narrower CT_{range}, higher thermal sensitivity, and higher maximum performance), and 2) an accumulation of temperature generalists in more thermally variable environments.

There was little evidence for tradeoffs in thermal performance traits (Fig. 4). While the fastest ant species were mostly fastest at 35°C, there were exceptions (Fig. 4a). For the other two possible tradeoffs (Fig. 4b, c), there was little evidence that ant species with broad thermal ranges were less temperature sensitive, or had lower maximum speeds. In both cases, plots of these tradeoff surfaces showed broad scatter, with slopes failing to differ from 0. As before, these relationships persisted under PIC.

In contrast, there was support for the prediction that the hotter, more variable canopy generates weaker temperature sensitivity (slope), lower $\text{rSpeed}_{\text{max}}$, and broader $\text{rCT}_{\text{range}}$ (Fig. 5). Consistent with the predictions of Thermal Adaptation, understory ants averaged TPCs that showed stronger, positive thermal sensitivity than canopy-nesting species (Fig. 5a). An understory species' average worker speed increased with temperature nearly twice as fast (0.015 vs. 0.027 cm^{-s}/°C, KW Chi-Square = 3.7, $P = 0.067$). Compared to canopy nesters with broader thermal ranges, ground-nesting ants were more dependent on temperature to achieve high speeds. Similarly, canopy ants had a lower maximum velocity for a given body size (Fig. 5b, $P = 0.053$, and were active across a broader range of surface temperatures for a given body size (Fig. 5c, $P = 0.0002$).

Phosphorus-tolerance hypothesis

Across 26 ant species (22 from the understory and four from the canopy) the fraction of P in ant tissue varied three fold (Fig. 6), from 3,166 ppm (for *Eciton hamatum*) to 9,542 ppm (for *Monomorium pharaonis*). Least squares regression revealed that as whole body phosphorus content increased, CT_{max} also increased (Fig 5c). Two other parts of the TPC differed in their response to tissue

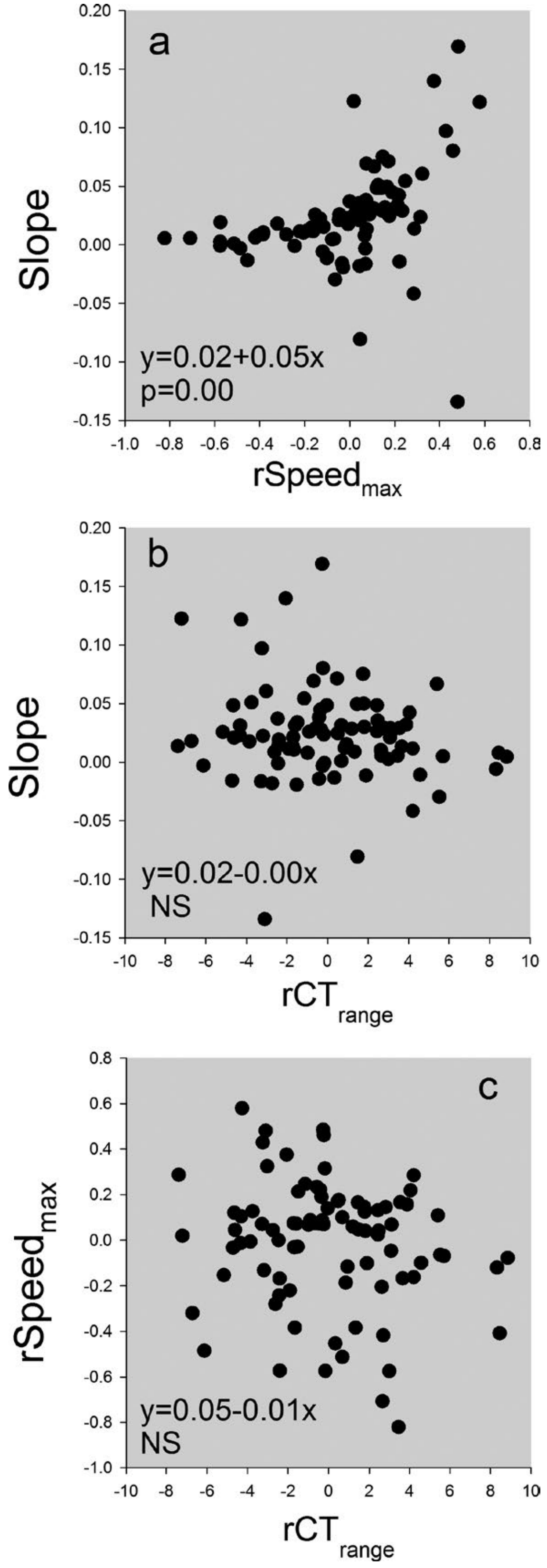

FIG. 4. Testing for interspecific tradeoffs among the three parameters describing ant thermal performance. Slope is the thermal sensitivity of speed, (i.e., the slope of speed vs. temperature from 20–35°C); $\text{rSpeed}_{\text{max}}$ is the mass corrected speed (the residual of body mass versus maximum speed, see Fig. 3); $\text{rCT}_{\text{range}}$ is mass corrected thermal range (i.e., the residual of body mass versus CT_{max}-CT_{min}). RMA regression results are embedded.

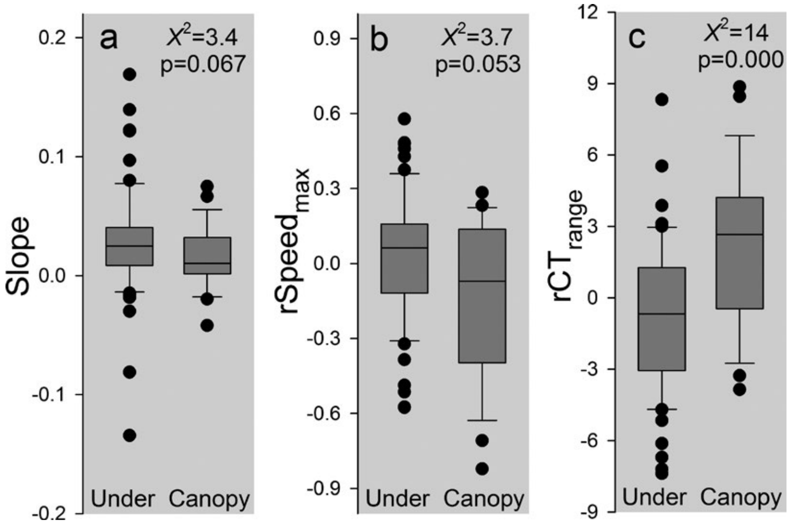

FIG. 5. Testing predictions of Thermal Adaptation hypothesis using populations from the hot and variable canopy versus the cooler, less variable understory. Three TPC parameters are compared, using a bar and whisker plot and comparing median values with a Wilcoxon X^2 value. (a) Slope is the thermal sensitivity of speed, (i.e., the slope of speed vs. temperature from 20–35°C). (b) rSpeed$_{max}$ is the mass corrected speed (i.e., the residual of body mass versus maximum speed, see Fig. 3). (c) rCT$_{range}$ is mass corrected thermal range (i.e., the residual of body mass versus CT$_{max}$-CT$_{min}$).

P. As P content increased, higher temperatures were less able to enhance speed (Fig. 5a). In contrast, there was no relationship between P content and maximum speed for a given body size (Fig. 5b). These relationships persisted under PIC.

DISCUSSION

The TPCs of 87 ant species of a Panamanian forest show a diversity of shapes: from the canonical "shark fin" gradual rise and sharp decline, to its converse. This diversity makes sense when we combine a strong hypothesis—Thermal Adaptation—with an underappreciated thermal gradient of surface temperatures—the hotter, more variable forest canopy versus the cooler, less variable shade below. The Thermal Adaptation hypothesis correctly predicts that the hotter canopy favors ant species with higher CT$_{max}$, and that the more variable canopy environments favor temperature generalists with a high thermal tolerance range but lower maximal speed. When we search for mechanisms underlying this diversity, we find no clear interspecific tradeoffs between thermal range, thermal sensitivity, and peak performance. We do, however, find evidence for the Phosphorus-Tolerance hypothesis: one-third of interspecific variation in CT$_{max}$ is associated with the phosphorus fraction in an ant's tissue.

Thermal adaptation and the absence of tradeoffs

Although Thermal Adaptation often uses the logic of tradeoffs (thermal generalists have low maximal performance due to the costly resources used to achieve it), we found little evidence for such tradeoffs in interspecific comparisons. In a synthesis of the literature, Angilletta (2009) also found few studies consistent with this assumption. The failure to find tradeoffs leads to a number of testable hypotheses. The first, and most basic, is that ant worker speed is inadequate as a fitness proxy for ant colonies. We found four quantitative studies that were consistent with a link between a colony's worker speed and a fitness proxy (Wilson 1976, Fewell 1988, Powell and Franks 2005, 2007). However, at least one (Pearce-Duvet et al. 2011) suggests that turning radius, not speed, is better correlated with discovery rates and presumably foraging efficiency. Given the large intra- and interspecific variation in foraging speeds, more such experiments would be useful to search for meaningful fitness proxies.

Second, natural selection can break tradeoffs by favoring different combinations of traits to promote coexistence (Rosenzweig 1995). Two of the fastest ants, species from the army ant genus *Labidus*, also showed the biggest differences in thermal sensitivities (from −0.13 to 0.10); two common species from the genus *Ectatomma* varied similarly (−0.2 to 0.07). In both cases, closely related, co-occurring species showed peak activity at lower and higher temperatures. The evolution of niches differences that may promote coexistence is another reason why studying communities of co-occurring species leads to valuable insights in thermal ecology (Rosenzweig 1995, Cerda et al. 1998, Huey et al. 2009).

A third, and related way to break tradeoffs is to break the assumption that the same resources must be invested in one or another ability. The ca. 400 species of ant on Barro Colorado Island vary in color, size, integument, pilosity, diet, and susceptibility to predators. If all these traits can shape an individual's thermal ecology, then it

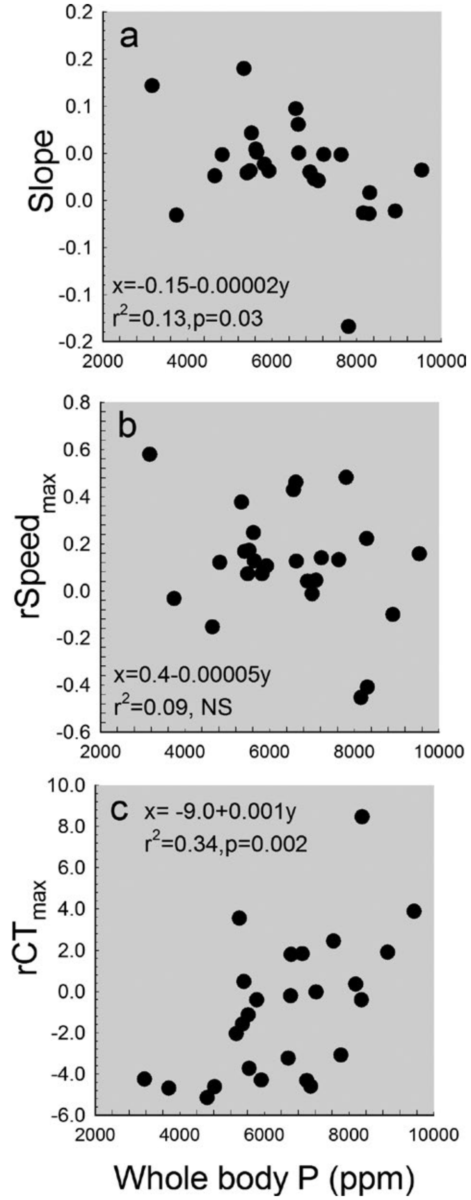

FIG. 6. Tests of the Phosphorus-Tolerance hypothesis for 26 Panama ant species. The concentration of P in ant tissue is related to (a) the thermal sensitivity of speed, (i.e., the slope of speed vs. temperature from 20–35°C) (b) $\mathrm{rSpeed}_{\max}$, the mass corrected speed (i.e., the residual of the maximum speed versus temperature, see Fig. 3); (c) $\mathrm{rCT}_{\max}$ the mass corrected thermal maximum (i.e., the residual of body mass versus $\mathrm{CT}_{\max}$).

is possible to build an individual with high thermal tolerance range and relatively high speed (e.g., *Pseudomyrmex* j_2, *Dolichoderus bispinosus*) by combining different traits. Outlier taxa in tradeoff scatterplots like those in Fig 4 are in this respect, highly instructive.

Phosphorus appears to promote thermal tolerance, CT_{max}

In the Phosphorus-Tolerance hypothesis we identified an essential macronutrient that appears to contribute to thermal tolerance in ants. Insects show a range of P, as glycerophosphate, in their haemolymph (Woods et al. 2002) and 170 insects ranging 4 orders of magnitude in size varied 5-fold in whole body P and 3.7-fold variation across the Hymenoptera (Woods et al. 2004). We find a three-fold variation in the fraction of P in the tissue of the 26 common ants tested. Many compounds and structures linked to metabolic and reproductive rates are high in P (Gillooly et al. 2005). Interestingly, one investment linked to high whole body P, mitochondrial density, has been posited to generate *low* thermal tolerance by increasing the ratio of O_2 demand to supply (Pörtner 2002): the resulting hypoxia at high temperatures, Pörtner posits, should result in system failure. If whole body P is associated with mitochondrial density, this would contradict the hypothesis.

What is the source of the three-fold variation in these 26 ant species? The simplest explanation for the correlation of P to $\mathrm{CT}_{\max}$ is that ants able to endure high thermal stress are more efficient at collecting and storing phosphorus. Phosphorus availability varies across the landscape (Walker and Syers 1976, Kaspari and Yanoviak 2008) and, in one study, the P content of an herbivorous weevil tracked that of the shrub on which it foraged (Schade et al. 2003). It would appear that the strongest P gradient in our study would be from canopy to understory, given that canopy ants are more herbivorous on average than those foraging in the brown food web below (Yanoviak and Kaspari 2000 and references therein) and animal tissue tends to have more P than plant tissue (Woods et al. 2004). This suggests that the hot canopy would be particularly deficient in the P, constraining, not allowing for high $\mathrm{CT}_{\max}$.

There is, however, one way that herbivores can achieve a high P diet: seeds are relatively high in P (reflecting their ribosome rich endosperm). Moreover, granivory in ants is a common syndrome, particularly in deserts but also in the rainforest understory (Kaspari 1993). We thus suggest the working hypothesis that our high-P ants are granivores.

Caveats on estimating TPCs

A variety of factors can introduce bias or noise when measuring TPCs. For example, our method using surface temperatures from 20–35°C may underestimate $\mathrm{Speed}_{\max}$ for canopy species relative to understory species if $\mathrm{Speed}_{\max}$ is found in the gap between 35°C and $\mathrm{CT}_{\max}$. We think this unlikely, given the decelerating speed of canopy ants from 27–35°C relative to understory ants.

Linearizing the speed*temperature curve likely simplified the shape of TPC for the 19 species (22%), with a top recorded speed at 27°C (Fig 2 a, b). Linearizing across the three temperatures may thus contribute to our failure to find a tradeoff (Fig 4), and may have eroded two patterns: the lower thermal sensitivities of canopy ants ($P = 0.067$, Fig. 5) and the decreasing thermal sensitivity of high P species ($P = 0.03$, Fig. 6).

It is also possible that canopy ants are slower for multiple reasons unrelated to thermal ecology. One possibility raised by a reviewer is that, as a misstep in the canopy has larger consequences than in the litter, canopy ants may be slower because they are more cautious. This is testable, but not yet tested.

Finally, our ramping protocol (27°C→20°C→CT_{min}→27°C→35°C→CT_{max}) showed evidence of decreased speeds for the fastest ants after they were returned to the second 27°C trial (second ~$first^{0.75}$). This, and the restricted size of the arena, likely caused us to underestimate top speed of our fastest ants. For example, we recorded *Eciton hamatum* 's top speed as 4 cm^{-s}; studies of free-living *E. hamatum* (Hurlbert et al. 2008) recorded speeds of up to 8 cm^{-s}.

Finally, some unaccounted variance may arise if some species are diurnal while others are nocturnal—nocturnal ants from the canopy and litter would presumably experience similar environments. Although previous studies of the BCI ant fauna found little such temporal partitioning (Kaspari and Weiser 2000) that study under sampled canopy ants. In provisional support of the prediction that nocturnal ants show more thermal sensitivity, we note that diurnal beetles in the Namib (experiencing 30–40°C) appear to have lower Q_{10}s (i.e., less thermal sensitivity) than their nocturnal counterparts (15–30°C, Lease et al. 2014).

The ubiquity of vertical clines in microclimate

The origins of the Thermal Adaptation hypothesis are rooted in Janzen's observations that the tropics are warmer and less seasonally variable than the temperate zone (Janzen 1967, Huey and Kingsolver 1993). But ecosystems are also composed of layers, with those closer to the sun exposed to the diurnal patterns of warming and cooling, with those below both cooler and less variable. There are numerous opportunities for such vertical, thermal partitioning. For example, as one travels downward from the soil surface, temperatures increasingly stabilize (Coleman and Crossley 1996). Likewise, in Earth's oceans, the abyssal depths are a cold and constant 2–3°C, while temperate surface waters can vary by 6°C over 24 h (Pacanowski and Philander 1981). We predict that whenever clades partition their environment vertically, there is the opportunity for thermal adaptation, with broad TPCs of temperature generalists above, and narrower temperature specialists below.

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