# Coping with the cold: minimum temperatures and thermal tolerances dominate the ecology of mountain ants

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**Abstract.** 1. Ants (Hymenoptera: Formicidae) are often cited as highly thermophilic and this has led to a range of studies investigating their thermal tolerances. It is unknown, however, if the geographic distribution of ant thermal tolerance conforms to the two major macropyhsiological rules that have been found in other taxa: Janzen's and Brett's rules. In addition, there is a paucity of data on how the lower thermal tolerances of ants are able to influence behaviour.

2. These two knowledge gaps were addressed here by sampling ants across a 1500 m elevational gradient in southern Africa and estimating the upper (CTmax) and lower (CTmin) thermal tolerances of 31 and 28 species, respectively. Ant abundances and soil temperatures were also recorded across the gradient over 6 years.

3. It was found that the average CTmin of the ants declined with elevation along with environmental temperatures. It was also found that the correlation between abundance and local temperature depended on the ant species' CTmin. The activity of species with a low CTmin was not constrained by temperature, whereas those with a high CTmin were limited by low temperatures.

4. For the first time, evidence is provided here that the thermal tolerances of ants are consistent with two major macrophysiological rules: Brett's rule and Janzen's rule. A mechanistic link between physiology, behaviour and the environment is also shown, which highlights that the ability of ants to deal with the cold may be a key, but often overlooked, factor allowing multiple ant species to succeed within an environment.

**Key words.** Ants, foraging, macrophysiology, mountains, temperature, thermal tolerances.

## Introduction

Temperature is important for all of life. It dictates the metabolic rate of organisms (Huey & Kingsolver, 1989), influences activity patterns and can impose controls on geographic distributions (Gaston & Chown, 1999; Jenkins & Hoffmann, 1999). If an environment is too hot or too cold for a species then it will not perform well and, potentially, will not be able to exist there at all. As a result, managing for extremes of temperature is critical for organisms and has led to a diversity of thermoregulatory adaptations (Angilletta, 2009). Globally, insects are one of the most diverse and functionally important groups of animals (Wilson, 1987) and have been characterised as 'thermal warriors', given that their lifestyle and success are so reliant on maintaining optimal temperatures (Heinrich, 1996). Understanding how the diversity of insect thermal tolerance is distributed across space, and the consequences this has for species and communities, is central to many basic and applied goals in ecology and entomology. Current and potential future insect geographic distributions, species interactions and ecosystem functions that they mediate are all linked to thermal tolerances (Heinrich, 1996; Chown & Nicolson, 2004).

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The ants (Hymenoptera: Formicidae) are a group of insects for which the broad patterns of diversity in thermal tolerance have only recently begun to be revealed (e.g. Diamond *et al.,* 2012; Kaspari *et al.,* 2015). Ants are abundant and ubiquitous on nearly all continents (Fisher, 2010), display a fascinating diversity of life-history strategies (Hölldobler & Wilson, 1990) and are believed to be functionally critical organisms in a range of environments (Evans *et al.,* 2011; Zelikova *et al.,* 2011; McGlynn & Poirson, 2012). Moreover, ants are commonly cited to be a thermophilic group (heat-loving; Hölldobler & Wilson, 1990; Kaspari *et al.,* 2000). Ant diversity increases with temperature at a range of spatial scales (Sanders *et al.,* 2007; Jenkins *et al.,* 2011; Bishop *et al.,* 2014) and temperature has been shown to positively influence ant running speed (Hurlbert *et al.,* 2008; Kaspari *et al.,* 2016). There are also a variety of both individual (Cerdá & Retana, 2000; Shi *et al.,* 2015) and colony-level (Kadochová & Frouz, 2013) thermoregulatory mechanisms that ants use to maintain optimum temperatures in both hot and cold conditions.

Despite the well-established link between many aspects of ant ecology and temperature, there are two key areas of ant thermal tolerance research that remain poorly understood. The first of these is whether broad spatial patterns in the diversity of ant thermal tolerance are consistent with those reported for other taxa. The second is our relative lack of knowledge on how lower thermal limits influence ant ecology.

There are two broad-scale geographic patterns in thermal tolerance that have emerged. Janzen's rule (Janzen, 1967), commonly called the climate variability hypothesis, states that greater variation in environmental temperatures is matched by a greater range in organismal thermal tolerances (Stevens, 1989; Gaston *et al.,* 2009). Brett's rule states that there is less geographic variation in upper than in lower thermal tolerances (Brett, 1956; Gaston *et al.,* 2009). A range of terrestrial verte-brate, invertebrate and plant species show patterns that match the predictions of Janzen's and Brett's rules (Addo-Bediako *et al.,* 2000; Sunday *et al.,* 2011; Araújo *et al.,* 2013; Hoffmann *et al.,* 2013). Organisms tend to have larger thermal tolerance ranges in environments known to be more variable and this is due to greater variation in lower thermal tolerance limits. For ants, it has been shown that there is little geographic variation in upper thermal limits (Diamond *et al.,* 2012), as measured by their crit-ical thermal maximum (CTmax). How the lower limits or the range of tolerance change over environmental gradients in ants is unknown.

The thermophilic characterisation of the ants has led to a number of studies that focus largely on their upper thermal limits and what they mean in the context of climate change. Perhaps unsurprisingly, hotter conditions tend to favour ants with a higher CTmax. This effect can be seen when comparing different microhabitats within the same ecosystem (Baudier *et al.,* 2015; Kaspari *et al.,* 2015), under experimental shade or heating regimes (Wittman *et al.,* 2010; Stuble *et al.,* 2013) and in the different daily activity rhythms of ant species (Fitzpatrick *et al.,* 2014). Similarly to other taxa (Sunday *et al.,* 2014), it appears that tropical lowland ant species may be the most physiologically susceptible to future climate warming (Diamond *et al.,* 2012).

What often goes unappreciated is that the thermophilic nature of ants must also mean that they are cryophobic (cold-fearing). Cool temperatures should also constrain ant activity and perfor-mance. The ant thermal tolerance literature, however, tends to focus on species and environments where extreme heat is more likely to be a limiting factor (Cerdá & Retana, 2000; Arnan & Blüthgen, 2015; Kaspari *et al.,* 2015). This is despite the fact that lower thermal tolerance may play a key role in main-taining global ant diversity and influencing species ranges. For example, in the Appalachian mountains of the USA, Warren and Chick (2013) showed that the critical thermal minimum temper-ature (CTmin) of the montane *Aphaenogaster picea* was consis-tently ~2 °C lower than that of the coastal *Aphaenogaster rudis.* As minimum temperatures rose in this region over a period of 40 years, the cold-intolerant *A. rudis* gained access to higher ele-vational sites and has now begun to displace the cold-tolerant *A. picea.* The CTmin of ants clearly has the ability to influence their demography and distribution but is a largely unexplored topic.

Here, we start to address these shortcomings in the ant thermal tolerance literature, while also testing for phylogenetic signal in tolerance measures. We investigate how both the CTmax and CTmin of ants change along a 1500 m elevational gradient in a bid to tackle three specific questions:

1  Do patterns of ant thermal tolerance conform to the predic-tions of Janzen's rule across elevation?
2  Do patterns of ant thermal tolerance conform to the predic-tions of Brett's rule across elevation?
3  Do the CTmin and CTmax of ants influence their foraging behaviour under different temperatures?

We predict: (i) that the thermal tolerance range will correlate with greater environmental temperature variability; (ii) that, in line with other taxa, lower thermal tolerance limits will vary much more than the upper limits over the elevational gradient; and (iii) that, in our mountain ecosystem, the CTmin of species, but not their CTmax, will impose constraints on the ability of species to forage under different temperature regimes.

## Materials and methods

### Study site

Field and experimental work took place in the Sani Pass of the Maloti-Drakensberg mountains. The Sani Pass is the only road running through the Maloti-Drakensberg Transfrontier Conservation Area of South Africa and Lesotho. The pass ranges in elevation from 1500 m above sea level (a.s.l.) (29°64′S 29°45′E) to 2874 m a.s.l. (29°60′S 29°29′E). This area is part of the grassland biome of southern Africa (Cowling *et al.,* 1997) and is recognised as a centre of endemism (Carbutt & Edwards, 2006; Kuhlmann, 2009).

### Live ant sampling

Live ants were sampled from four different elevations (1500, 1800, 2400 and 3000 m a.s.l.) in January and February 2014

using a combination of baits (sugar water and cat food) and active searching. Ants were transported back to the laboratory and kept in perforated containers with soil and a damp piece of cotton wool. The thermal tolerances of the live ants were tested within 24 h of their collection from the field. We do not know how many colonies were sampled for each species and this remains a caveat of this research.

*Thermal tolerance experiments*

Thermal tolerances were measured as the ants' CTmax and CTmin. Individual ants experienced only a single experimental run (either CTmin or CTmax) and were then were not subjected to further testing. A dry heat bath (Tropicooler 260014-2, Boekel Scientific, Feasterville, PA, USA) was used to estimate CTmax and CTmin. The heat bath has a temperature range of $-19$ to $69\,°C$ with an accuracy of $\pm 1\,°C$. The heat bath contains two wells of $8.7 \times 5.7 \times 3.7\,cm^3$ into which fits an aluminium heating block. Each heating block has 14 wells, each of which holds a single 1.5 ml microcentrifuge tube. At full capacity, a single experiment could test 28 individual ants (one ant per tube $\times$ two blocks $\times$ 14 tubes $= 28$).

An experimental run testing either CTmin or CTmax was done as follows. A single worker was placed into a microcentrifuge tube. The microcentrifuge tube was plugged with a small piece of cotton wool to prevent the worker ant from climbing to the top of the tube and finding a thermal refuge and placed into one of the aluminium heating blocks within the heat bath unit. Ants were acclimatised for 15 min at 15 or $25\,°C$ for CTmin and CTmax, respectively. After this, the temperature was lowered (CTmin) or raised (CTmax) by $1\,°C$. The heat bath was kept at the new temperature for 3 min. The ants were then checked for the loss of muscle coordination (Ctmax; Lutterschmidt & Hutchison, 1997) or the absence of any movement at all (Ctmin; Hazell & Bale, 2011) by quickly removing and flicking the individual microcentrifuge tubes. If an individual ant met these criteria, this was deemed to be their critical temperature and was recorded. The experimental run stopped once all individuals had reached their critical temperatures.

Individuals of different species were randomly assigned to wells and positions within the aluminium heating blocks and heat bath unit for each experimental run. This was to ensure that no systematic bias was introduced in the event that different parts of the heat bath unit heated or cooled at different rates. Where possible, we repeated each experiment (CTmin and CTmax) three times for each species from each elevation. During each run of an experiment, there were five individuals of each species that were being tested at that time. Consequently, 30 individuals of a species were tested from a single elevation when they could be found in sufficient numbers (five individuals $\times$ three replicates $\times$ two experiments $= 30$).

We recognise the range of pitfalls associated with determining insect physiological tolerances experimentally – including the effects that the rate of temperature ramping may have on the results (Terblanche *et al.,* 2011). We emphasise the logistical constraints imposed on performing these experiments with field-caught animals and argue that our thermal tolerance estimates are comparable within this study but caution against direct numerical comparison with other insect (including ant) thermal tolerance results which have used different experimental details and approaches.

*Time series data*

Data on forager abundance/activity were obtained by sampling epigaeic (ground-dwelling) ants biannually for 6 years from January 2006 to September 2012. The two sampling periods in each year represent the hotter and wetter season (January) and the colder and drier season (September/October). Two replicate blocks, spaced at least 300 m apart, were established at four different elevations. The four elevations were at 1500, 1800, 2400 and 3000 m a.s.l., the same as those which were sampled for live ants. At each block, 10 pitfall traps were set in two parallel lines with 10 m separating each adjacent trap. Traps were 150 ml in volume with a diameter of 55 mm and a depth of 70 mm. Rain guards supported on wire legs were placed over each trap to prevent flooding. All traps contained a 50% ethylene glycol preservative and were left out for five trapping nights in total. Traps were checked and replaced every 2–3 days to prevent overfilling. Ants were transferred to 70% ethanol in the laboratory and identified to morphospecies or species level where possible. These ant abundance data are a subset of those described and analysed in Bishop *et al.* (2014) and Bishop *et al.* (2015). In those studies, data from an additional two replicate blocks at each elevation and four more elevational sites were analysed. This study only analyses time series data from replicates for which we had iButton data and elevational sites where we sampled live ants for the thermal tolerances.

Thermocron iButtons (DS1921G, Semiconductor Corporation, Dallas/Maxim, TX, USA) were used to record soil temperatures through time at each replicate block. The iButtons were buried 10 mm below the soil surface and recorded the temperature every 1.5 h. From January 2010, hourly readings were taken as a higher capacity iButton (DS1922L) was phased into use. The iButton data were inspected for cases where the unit had clearly malfunctioned or been directly exposed to the sun. These cases were removed from the temperature time series before analysis.

*Phylogenetic signal*

A genus level, time-calibrated phylogeny from Moreau and Bell (2013) was used to estimate phylogenetic signal in CTmin and CTmax. This was done in two ways. First, we added species from this study as polytomies onto the original genus phylogeny and the calculated phylogenetic signal. Secondly, we calculated genus level means in the thermal tolerance measures and used the original genus level phylogeny to calculate phylogenetic signal. Two genera, *Lepisiota* and *Streblognathus*, were not present on the original phylogeny. These genera were inserted as tips next to their closest relative. *Lepisiota* was inserted as a sister to *Plagiolepis* (Ward *et al.,* 2016), and *Streblognathus* was inserted as a sister to *Odontomachus (*Schmidt, 2013*)*. Phylogenetic signal was calculated using Pagel's $\lambda$ (Pagel, 1999)

and Blomberg's *K* (Blomberg *et al.,* 2003). A likelihood ratio test was used to test for a significant departure of both of these statistics from 0 (no phylogenetic signal). The *phytools* package in R was used to manipulate the phylogeny and perform the phylogenetic signal tests (Revell, 2012).

### Temperature and elevation (Aim 1)

The minimum, maximum and variance in temperatures were calculated for January (the month during which our thermal tolerance sampling and experiments took place) for each elevational site from the iButton data logger time series over the years 2006–2012 inclusive. Differences in these temperature variables across elevation were not statistically analysed due to temporal pseudoreplication. There were only two true (spatial) replicates per elevation.

### Thermal tolerance and elevation (Aims 1 and 2)

Differences in the average thermal tolerances of the ants between elevations were tested using linear mixed models with species as a random effect. Species were included multiple times with different thermal tolerance estimates from each elevation from which they were found. Consequently, each data point is a species/elevation combination. No time series data were incorporated into this analysis. Average CTmin and CTmax for each species within each elevation were calculated from the individual level data. CTrange was calculated as the difference between CTmax and CTmin for each species within each elevation. Three separate mixed-model ANOVA analyses were used to test for differences in CTmin, CTmax and CTrange across the four elevational classes. Chi-squared likelihood ratio tests were used to assess the significance of elevation in each case. If significant, Tukey's honest significant difference (HSD) tests were performed to reveal which elevations were different from each other. Tukey's HSD tests were run using the *glht* function in the multcomp package in R (Hothorn *et al.,* 2008). We did not perform phylogenetically controlled analyses as there was no phylogenetic signal in the thermal tolerance traits (see the Results). Marginal ($R_m^2$, fixed effects) and conditional ($R_c^2$, fixed + random effects) $R^2$ values were calculated (Nakagawa & Schielzeth, 2013). Mixed models were built using the LME4 package (Bates *et al.,* 2014) and tested for significance using the ANOVA function.

### Thermal tolerance and foraging behaviour (Aim 3)

To assess how thermal tolerance is related to foraging behaviour, we first calculated a species-specific temperature response. The temperature response was defined as the Pearson's correlation coefficient between log-transformed abundance and temperature for each species (Fig. 1a) over the time series (Fig. 1b). Abundance was log-transformed to achieve normality. A positive correlation coefficient indicates that a species is more abundant at higher temperatures. Temperature was calculated as the mean, minimum and maximum temperatures at each

replicate block (two replicates × four elevations = eight replicates) during all of the pitfall sampling periods (7 years × two seasons = 14 time periods). We excluded species from the analysis if they were not detected in three or more sampling periods. We also excluded time periods for which a given species had an abundance of zero, as we were interested in how abundances changed with temperature rather than if occurrences were affected.

We related variation in species' temperature response to their CTmin and CTmax (Fig. 1c,d) using linear mixed models. This led to six models as the temperature response was calculated three times using different temperature data (mean, minimum and maximum). Species that were collected at multiple elevations were treated as separate 'species' in the analysis to allow incorporation of the different thermal tolerance and temperature response estimates that we gathered at different elevations. Consequently, species was used a random effect. The models were weighted by the number of data points that were used to calculate the individual temperature responses. This weighting was done so that we could include data from as many species as possible whilst also recognising that some were only caught in a small number of time periods. Chi-squared likelihood ratio tests were used to assess the significance of the critical temperature (CTmin or CTmax) in explaining variation in the temperature response. Marginal and conditional $R^2$ values were also calculated.

All data manipulation and analyses were performed in the R statistical environment (R Core Team, 2014).

## Results

We collected CTmin estimates for 28 species and CTmax estimates for 31 species (Appendix S1). We could not collect CTmin for three species. Thermal tolerance was estimated at more than one elevation for eight and nine species for CTmin and CTmax, respectively. The total size of the species pool was 92 (Bishop *et al.,* 2014). Consequently, we sampled 33% of the available fauna. The sample of species for which we gathered thermal tolerance data was, as expected, biased toward the more common species. After calculating rank abundances on the entire species pool using the time-series data (Appendix S2), the subset for which we had thermal tolerance data had an average rank of 25.42 (in contrast to the average rank of 46.5 for the entire fauna). The most common species for which we had thermal tolerance data was ranked first and the least common was ranked 76th (out of 92).

### Phylogenetic signal

Using the species level polytomy tree, neither CTmin (Pagel's $\lambda = 0.21$, $P = 0.39$, Blomberg's K $< 0.01$, $P = 0.26$) nor CTmax (Pagel's $\lambda < 0.01$, $P = 1$, Blomberg's K $< 0.01$, $P = 0.42$) displayed significant phylogenetic signal. Similar results were found for CTmin (Pagel's $\lambda = 0.21$, $P = 0.49$, Blomberg's K $= 0.59$, $P = 0.57$) and CTmax (Pagel's $\lambda < 0.01$, $P = 1$, Blomberg's K $= 0.64$, $P = 0.43$) on the genus-level phylogeny using genus averages.
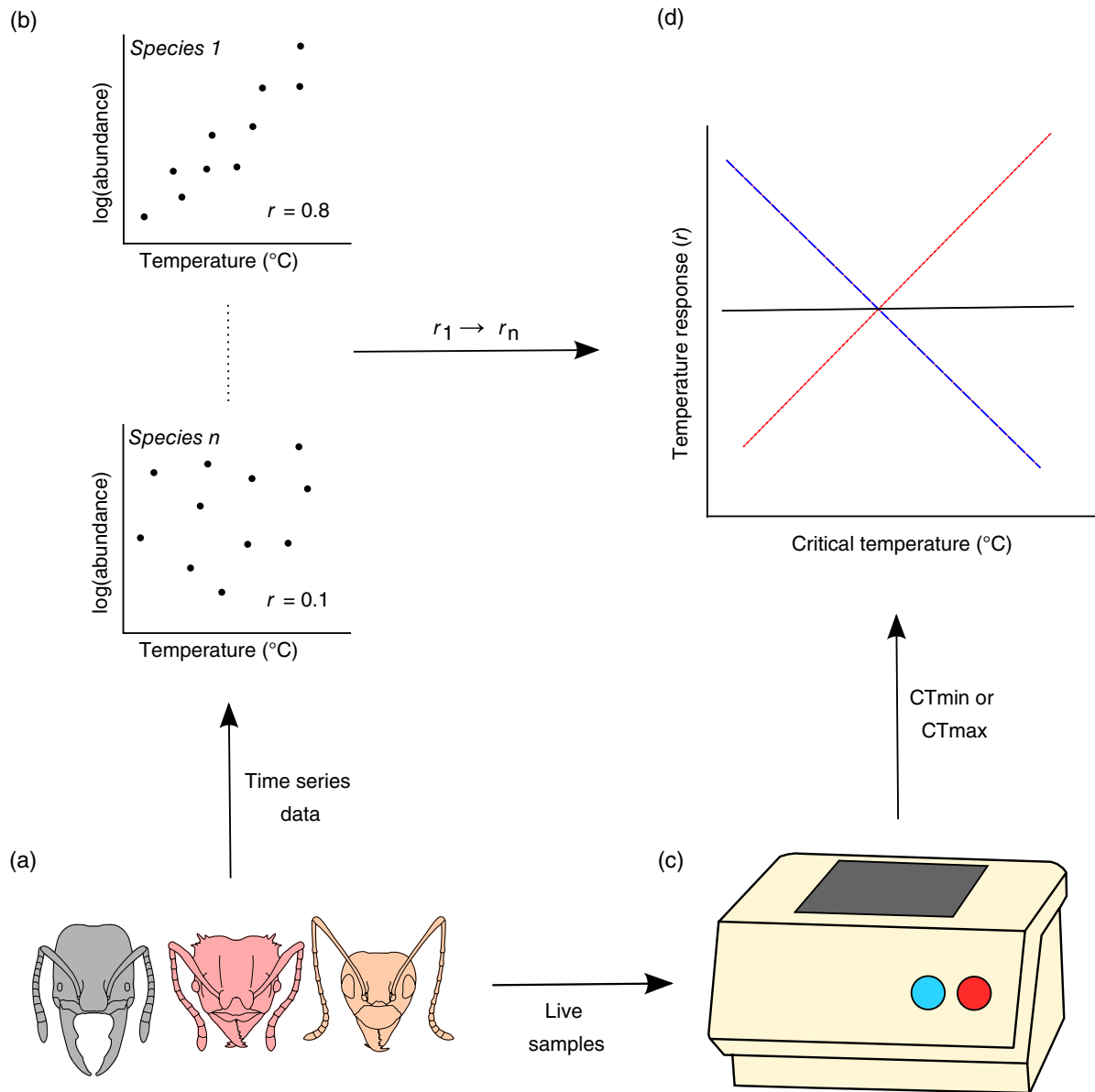
(b)



(d)



**Fig. 1.** Schematic illustrating the analysis for relating thermal tolerance and foraging behaviour. (a) Multiple ant species are collected from the Sani Pass. (b) The correlation between abundance (pitfall trapping over 7 years, 2006–2012) and temperature (obtained from data loggers) is calculated for each species and within each elevational band. This can be done using minimum, mean or maximum environmental temperatures. (c) Critical thermal maxima (CTmax) and minima (CTmin) are estimated from live specimens collected in 2014 using a dry heat bath. (d) The temperature responses, i.e. the correlation coefficients from (a), are regressed against the critical temperature values, obtained from (c). Each data point in (d) is a species from a single elevation. Lines in (d) represent three theoretically different possible relationships between the temperature response and the species' critical temperatures. Solid black, no relationship; dashed blue, stronger response with low critical temperature; dotted red, stronger response with low critical temperature. [Colour figure can be viewed at wileyonlinelibrary.com].

*Temperature and elevation (Aim 1)*

Minimum temperatures clearly differed across elevation. The average minimum temperature declined from 14.7 °C at 1500 m a.s.l. to 5.9 °C at 3000 m a.s.l. (Fig. 2a). The maximum and variance in temperature showed much greater overlap between elevations than did the minimum temperature (Fig. 2b,c). Maximum temperature tended to decline with increasing elevation (from 34.9 to 26.7 °C) but to a lesser extent than minimum temperature. Variance in temperature showed no clear trend with elevation (Fig. 2c).

*Thermal tolerance and elevation (Aims 1 and 2)*

The values of CTmin differed significantly across elevation (mixed-effects ANOVA, df = 3, $\chi^2 = 27.74$, $P < 0.01$, $R_m^2 = 0.52$,
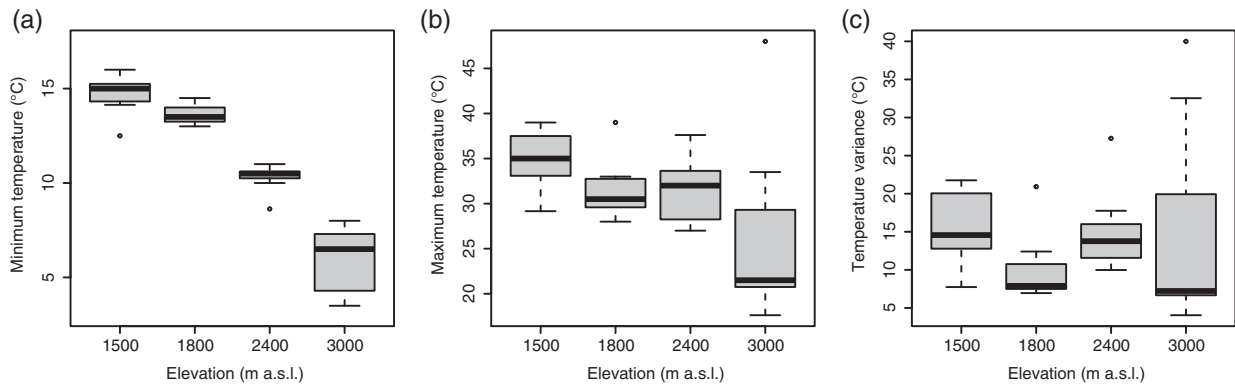
**Fig. 2.** Box plots showing minimum (a), maximum (b) and variance (c) in temperature in January across different elevations in the Sani Pass of the Maloti-Drakensberg Mountains, southern Africa. Temperatures are taken from data loggers placed in the soil. Box plots show the median (central band), 25th and 75th percentiles (bottom and top of boxes) and 1.5 times the interquartile range above or below the 25th and 75th percentiles (whiskers). A single data point is a temperature estimate from one replicate during 1 year, and consequently there are a maximum of 14 data points for each box (two data loggers × 7 years = 14). m a.s.l., m above sea level.

$R_c^2 = 0.71$, Fig. 3a). Tukey HSD tests revealed that these differences were between 1500 and 2400 m a.s.l. ($p < 0.01$), 1500 and 3000 m a.s.l. ($p < 0.01$), 1800 and 2400 m a.s.l. ($p < 0.01$), and 1800 and 3000 m a.s.l. ($p < 0.01$). This splits the elevations into two groups. CTmin was significantly lower at 2400 and 3000 m a.s.l. than at 1500 and 1800 m a.s.l. CTmax (mixed effects ANOVA, df = 3, $\chi^2 = 2.60$, $P = 0.46$, $R_m^2 = 0.06$, $R_c^2 = 0.72$, Fig. 3b) and CTrange (mixed effects ANOVA, df = 3, $\chi^2 = 6.6$, $P = 0.09$, $R_m^2 = 0.17$, $R_c^2 = 0.66$, Fig. 3c) did not differ across elevation but both tended to increase with increasing elevation.

*Thermal tolerance and foraging behaviour (Aim 3)*

After filtering species with insufficient time series data (see Methods), 26 species/elevation combinations were used for CTmin and 29 were used for CTmax. CTmax was not a significant predictor of any of the temperature response variables (minimum, mean or maximum, $p > 0.05$). There was a significant positive relationship between CTmin and the temperature response when using minimum temperatures (linear mixed model, d.f. = 1, $\chi^2 = 3.91$, $P = 0.048$, $R_m^2 = 0.026$, $R_c^2 = 0.033$, simple linear regression $R^2 = 0.25$, Fig. 4). Species with a low CTmin did not respond strongly to changes in minimum temperature. Those with a high CTmin tended to increase their abundances with increasing minimum temperatures. There was no significant relationship between CTmin and the temperature response when using mean or maximum temperatures.

**Discussion**

We provide evidence that the thermal tolerances of ants are consistent with two major macrophysiological rules: Brett's rule and Janzen's rule. Whilst previous studies have begun to reveal the diversity present in ant thermal tolerances, it has not been clear how this diversity may be structured geographically. We also found that the foraging behaviour of ants under different

temperatures can be mediated by their CTmin, but not their CTmax. Combined, these results show that there is not only more spatially structured variation in lower thermal limits in ants, but that this variation is able to control a key aspect of their ecology.

It is important to note that there is no phylogenetic signal in our estimates of CTmin or CTmax. Consequently, our main analyses did not control for any phylogenetic effects. This is in contrast to Diamond *et al.* (2012), who found small, but significant, phylogenetic signal in CTmax for ants in their global dataset. Their data show that closely related species had CTmax estimates that were more dissimilar to each other than expected under Brownian motion. The lack of any statistically signficant signal in our study is probably due to the relatively small sample size of 28 species, compared with the 156 species analysed by Diamond *et al.* (2012).

Whilst we find evidence that is consistent with both Brett's rule and Janzen's rule, the level of support differs for each. This is probably due to the nature of the temperature gradient that we have sampled. Our results fully support Brett's rule that there is greater geographic variation in lower than in upper thermal tolerance limits (Brett, 1956; Gaston *et al.,* 2009). This finding supports our original prediction (2). CTmin, but not CTmax, significantly changes with elevation (Fig. 3a,b). At higher elevations, ants tend to have a lower CTmin. This makes sense in terms of the environmental temperatures recorded at the different elevations. High elevations have a much lower minimum temperature compared with low-elevation sites, but the change in maximum temperature is not as pronounced (Fig. 2a,b). This asymmetric change in both tolerance limits and environmental temperature extremes, especially minimum temperature, mirrors that which is often reported across latitudinal gradients (Addo-Bediako *et al.,* 2000; Hoffmann *et al.,* 2013). Interestingly, CTmax and CTrange do tend to increase with increasing elevation. These results are nonsignificant but they may be driven by a combination of scarce resources and rapidly changing small-scale temperatures. We speculate that the high elevation ants may have to forage for longer periods and therefore
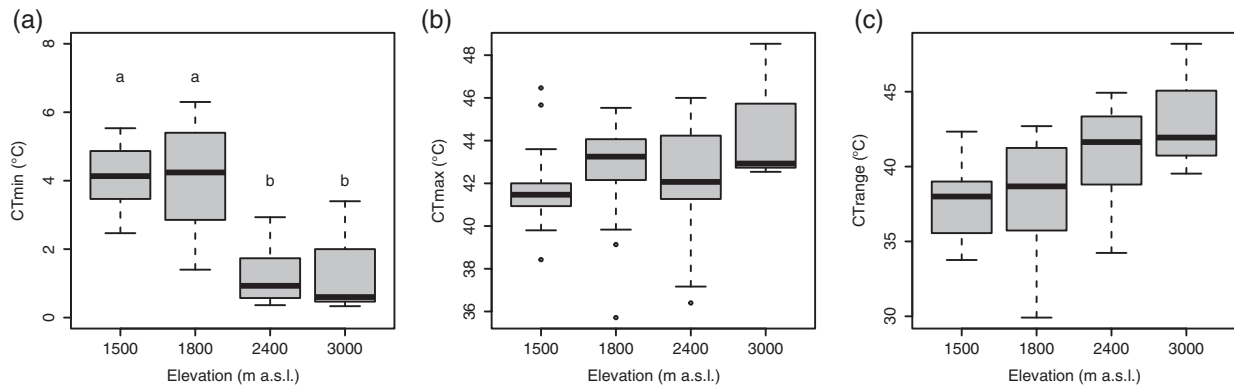
**Fig. 3.** Box plotsshowing CTmin (a), CTmax (b) and CTrange (c) (calculated as the difference between CTmax and CTmin) at different elevations in the Sani Pass of the Maloti-Drakensberg mountains, southern Africa. In (a), letters above boxes indicate significantly different groupings. Box plots show the median (central band), 25th and 75th percentiles (bottom and top of boxes) and 1.5 times the interquartile range above or below the 25th and 75th percentiles (whiskers). Each data point is a species. In all there were 37 observations of 28 species in (a), 41 observations of 31 species in (b) and 37 observations of 28 species in (c). m a.s.l., m above sea level.
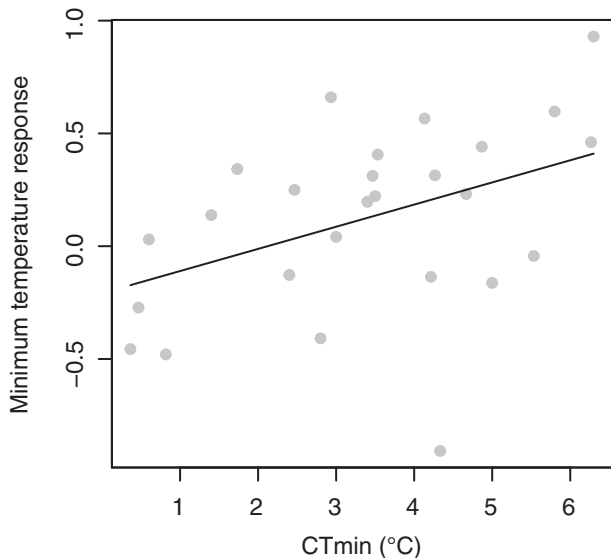


**Fig. 4.** Plot showing the relationship between species' minimum temperature responses and their critical thermal minimum (CTmin). The temperature response is defined as the Pearson's correlation coefficient between log-transformed abundance and (in this case) minimum temperature for each species over the time series. The fitted line is based on linear mixed-model predictions.

be forced to experience short bursts of high temperatures which they may ordinarily avoid. This effect may be exaggerated by the greater proportion of rocky ground at the highest elevations. We do not have the data to address this idea properly here.

Our data are consistent with, but do not fully support or discard, Janzen's rule. Janzen's rule predicts a greater thermal tolerance range where there is a more variable temperature environment (Janzen, 1967; Gaston *et al.*, 2009). We find that neither thermal tolerance range (Fig. 3c) nor variation in environmental temperature changes significantly across elevation (Fig. 2c). This does not support our original prediction (1). This

conclusion is at odds with a previous study, which used part of the same elevational gradient used here. Gaston and Chown (1999) found evidence for both Brett's and Janzen's rule in dung beetles. Their data show that the range in temperature does not change between 1500 and 3000 m a.s.l. (from an interpolated climatic surface, see Fig. 3 in Gaston & Chown, 1999); we find the same in our study using data loggers. For Gaston and Chown (1999), it is their inclusion of sites close to sea level that reveals increases in both temperature variation and thermal tolerance range with elevation. This implies that the collection of ant thermal tolerance data from a more extensive elevational gradient may reveal stronger evidence in support of Janzen's rule. We cannot unequivocally support or discard the rule in this case.

We find that CTmin mediates the relationship that species have with temperature (Fig. 4), but that CTmax does not. This is in line with our original prediction (3). A lower CTmin means that species' abundances are less affected by changes in temperature. This is most evident when comparing the relationship between abundance and minimum temperature with CTmin (Fig. 4). The temperature response variable indicates whether abundances correlate positively (>0) or negatively (<0) with increasing temperature. Figure 4 shows that species that respond negatively or are invariant to minimum temperatures have a low CTmin. Species with a high CTmin, on the other hand, respond positively to increasing minimum temperatures.

This pattern, that foraging activity is constrained by the link between temperature and species' physiological tolerances, is perhaps not surprising given the widespread understanding that ants are generally thermophilic (Hölldobler & Wilson, 1990). This is one of the first times, however, that a mechanistic link between physiology, behaviour and the environment has been made for ants. In the fauna we have sampled here, it is the CTmin of species and the coldest environmental temperatures that appear to determine how many foragers are active. Previously, it has been shown that ants change their foraging abundances over a range of timescales and that this is often linked to concurrent changes in temperature (Andersen, 1983; Fellers, 1989; Cerdá *et al.*, 1997; Dunn *et al.*, 2007).

Extreme temperatures are viewed as marginal environments, in which only subdominant or subordinate species will forage (e.g. Cerdá *et al.,* 1997). The data we present here suggest that these differences in preferred foraging times may be underpinned by differences in species' physiology. This conclusion can help us to better understand the current and future distributions of ants and their interactions with each other (Warren & Chick, 2013).

These results linking physiology to foraging behaviour are based on a relatively small sample size (26 species) yet we still detect significant effects. We argue that this is an impressive signal given the inherent noise present in abundance data. It is likely that factors such as disturbance (Andersen *et al.,* 2014), rainfall (Holway, 1998; Kaspari & Valone, 2002) and competitive interactions (Parr & Gibb, 2010) have left their mark on the abundance data of each species. Indeed, the abundances of species themselves, and consequently their foraging behaviours, may be influenced by a trade-off between thermal tolerance and dominance (Cerdá *et al.,* 1998). Our data are not at the right spatial or temporal scale to properly examine this trade-off, but we do not find a strong link between species abundance rank and thermal tolerance (Appendix S3). A next step in this research would be to evaluate the physiology-foraging link under controlled laboratory conditions and to also investigate a larger number of species over a larger area (elevation and temperature range) whilst also recording detailed environmental information.

We found no link between CTmax and forager abundances. In contrast, Stuble *et al.* (2013) reported that species with a higher CTmax were found foraging most at higher temperatures. We suspect that the CTmax plays a smaller role in our analyses because it is never actually consistently hot enough at our field site over our 5-day sampling periods. The study by Stuble *et al.* (2013), in contrast, concerns an experimental warming treatment. Comparing the influence of CTmin and CTmax on foraging behaviour in a number of different locations, and recording the influence this has on colony performance, would be a fruitful step forward for the field of ant thermal tolerance research.

Finally, we emphasise that further work understanding fine-scale variation in thermal tolerance, behaviour and colony performance over extensive gradients would be useful. We have begun to reveal patterns of thermal tolerance variation with this study, but extending the range of thermal environments sampled and increasing the sample size would be beneficial. For example, we find what looks like a step change in CTmin (Fig. 3a) but this is probably an artefact of our sampling regime. Sampling more species and individuals would allow much more scope to assess the relative importance of inter- versus intraspecific variation and also strengthen any conclusions that can be made in relation to behaviour and performance.

In summary, we find that patterns of ant thermal tolerance partly conform to existing macrophysiological rules. We find greater geographic variation in CTmin than in CTmax, which supports Brett's rule. Our data are consistent with Janzen's rule but we are unable to truly comment on it, given the lack of gradient in temperature variation in our data. Finally, we show that the physiology of ants, in particular their CTmin, imposes constraints on their ability to forage at different temperatures. This effect may have widespread consequences for our understanding of contemporary ant species diversity and coexistence and for the way in which they may change in the future.

## Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12364

**Appendix S1**. Thermal tolerances of ant species in the Sani Pass.

**Appendix S2**. Rank abundance plot of Sani Pass ants.

**Appendix S3**. Rank abundance vs thermal tolerance for Sani Pass ants.

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