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Can temperate insects take the heat? A case study of the physiological and behavioural responses in a common ant, *Iridomyrmex purpureus* (Formicidae), with potential climate change



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ABSTRACT

Insects in temperate regions are predicted to be at low risk of climate change relative to tropical species. However, these assumptions have generally been poorly examined in all regions, and such forecasting fails to account for microclimatic variation and behavioural optimisation. Here, we test how a population of the dominant ant species, *Iridomyrmex purpureus*, from temperate Australia responds to thermal stress. We show that ants regularly forage for short periods (minutes) at soil temperatures well above their upper thermal limits (upper lethal temperature = 45.8 ± 1.3 °C; CT_{max} = 46.1 °C) determined over slightly longer periods (hours) and do not show any signs of a classic thermal performance curve in voluntary locomotion across soil surface temperatures of 18.6–57°C (equating to a body temperature of 24.5–43.1 °C). Although ants were present all year round, and dynamically altered several aspects of their thermal biology to cope with low temperatures and seasonal variation, temperature-dependence of running speed remained invariant and ants were unable to elevate high temperature tolerance using plastic responses. Measurements of microclimate temperature were higher than ant body temperatures during the hottest part of the day, but exhibited a stronger relationship with each other than air temperatures from the closest weather station. Generally close associations of ant activity and performance with microclimatic conditions, possibly to maximise foraging times, suggest *I. purpureus* displays highly opportunistic thermal responses and readily adjusts behaviour to cope with high trail temperatures. Increasing frequency or duration of high temperatures is therefore likely to result in an immediate reduction in foraging efficiency. In summary, these results suggest that (1) soil-dwelling temperate insect populations may be at higher risks of thermal stress with increased frequency or duration of high temperatures resulting from climate change than previously thought, however, behavioural cues may be able to compensate to some extent; and (2) indices of climate change-related thermal stress, warming tolerance and thermal safety margin, are strongly influenced by the scale of climate metrics employed.

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1. Introduction

Climate change is one of the most pressing scientific issues facing humanity (AAS, 2010). Across Australia, average daily maximum temperatures have increased by 0.75 °C since 1910 (CSIRO-ABM, 2012) and future predictions are for a generally warmer and drier continent by 2030 (CSIRO, 2007). The likely impacts of climate change are expected to be complex and highly variable across Australia and world-wide (Walther et al., 2002). This may lead to a larger number of extreme maximum temperature events, but fewer and more intense minimum temperature extremes (WMO, 2011; Hansen et al., 2012). Major challenges are to predict

how organisms will respond to such changes and to identify species at risk of thermal stress, population decline and possible extinction (Root et al., 2003; Deutsch et al., 2008; Huey et al., 2009; Chown et al., 2010; Diamond et al., 2012; Andrew and Terblanche, 2013), and how this may impact on ecosystem function.

Ants are one of the most ubiquitous animals in terrestrial ecosystems and play crucial roles in ecosystem functioning on all continents except Antarctica (Lach et al., 2010; CAS, 2012). The essential role that ants play in natural ecosystems, including predation, seed dispersal, pollination, nutrient recycling, herbivore 'farming' and as a food source for other invertebrates and vertebrates (Lach et al., 2010), will likely be modified by a changing climate. Therefore understanding how ants will respond to climate variation is of fundamental importance in understanding, and sustaining, global biodiversity. Ants play a key role in providing

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ecosystem services and are habitat engineers making them flagship taxa to identify responses to changing abiotic factors (Gaston, 2011).

Ectothermic insects, particularly ants in the tropics have been argued to be more susceptible to climate warming due to their relatively low warming tolerance (WT) than more temperate or high latitude species (Deutsch et al., 2008; Diamond et al., 2012). This pattern is largely a result of the fact that upper temperature limits for survival and activity are relatively constant across the planet (e.g. Addo-Bediako et al., 2000; Deutsch et al., 2008; Overgaard et al., 2011; Sunday et al., 2011; Kellermann et al., 2012), and tropical species live in environments that are warmer and are therefore closer to their upper thermal limits and optimal performance temperatures (Topt). In addition, plasticity of tolerance may be intrinsically coupled to basal tolerance levels, further complicating prediction and forecasting efforts (e.g. Stillman, 2003; Calosi et al., 2008; Nyamukondiwa et al., 2011). For example, Stillman (2003) found that tolerance to higher temperatures may come at the expense of acclimation capacity in marine invertebrates. By contrast, among *Drosophila* species, Nyamukondiwa et al. (2011) found little evidence for trade-offs between basal high temperature tolerance and acute plasticity thereof, and instead, they found the exact opposite pattern (more plasticity accompanied more tolerance) (but see also Sgro et al., 2010 for intraspecific patterns).

Recently, several of the thermal stress risk generalisations have been questioned on the basis of microsite opportunities which are typically not accounted for in models of thermal stress and extinction risk (e.g. Sinervo et al., 2010; Clusella-Trullas and Chown, 2011; Sinervo et al., 2011), and that several ectotherm groups are potentially at greater risk in sites further from the tropics (e.g. Clusella-Trullas et al., 2011; Kellermann et al., 2012). In addition, exposure to different sub-lethal temperatures for a range of time durations might enhance ants survival over short time-scales (e.g. during foraging for a few minutes up to 4.5 h: Jumbam et al., 2008).

However, several testable predictions have generally been poorly examined. Furthermore, there has been a lack of information incorporating physiological tolerances with microclimatic and behavioural optimisation of insects in the field in assessing their risk to climate change, and the data for tropical and temperate zone species is, in some cases, notably sparse (see e.g. Kearney et al., 2009; Clusella-Trullas et al., 2011; Bonebrake and Deutsch, 2012). Both warming tolerance and thermal safety margins (TSM) are important indices as they characterise the geographic covariances of thermal performance curves (reviewed in Angilletta, 2009) and climate: warming tolerance is the difference between upper thermal tolerance and habitat ambient temperature and therefore defines how much warming an ectotherm can tolerate before performance is reduced to lethal levels; thermal safety margins is the difference between an organism's thermal optimum for performance and the current habitat ambient temperature and therefore gives an indication of how close animals thermal optima are to the current climatic temperature within their environment (Deutsch et al., 2008). While it is generally clear that different estimates of climate can influence these indices in profound ways (Clusella-Trullas et al., 2011; Diamond et al., 2012; Huey et al., 2009), few studies have considered microclimatic data at ecologically relevant times or spatial scales for ants (e.g. temperatures at the main foraging times on ant trails throughout the hottest months; differences between open and shaded habitats along foraging routes, and changes at different depths and galleries of the nest), and systematically examined the influence of these variables on estimates of susceptibility to climate change (though see e.g. Clusella-Trullas et al., 2011).

In Australia, one of the most common animal taxa, and with the highest biomass of all vertebrate and invertebrate taxa, are meat

ants (*Iridomyrmex* spp.) (Andersen, 2000). Relative to the phylogenetic and climatic diversity occupied by ant species worldwide it is perhaps surprising that there is not more research on their physiological responses to climate change (though for research on other ant species see e.g. Diamond et al., 2012; Jumbam et al., 2008; Maysov and Kipyatkov, 2009; Jayatilaka et al., 2011; Oberg et al., 2012; Ribeiro et al., 2012), and a particular dearth of information on the responses of Australian native insect fauna (but see Christian and Morton, 1992; Jayatilaka et al., 2011). Temperature also plays an important role in ant locomotion (Shapley, 1920, 1924; Greenaway, 1981; Hurlbert et al., 2008) by determining activity times and absolute speed (Cerda et al., 1998) but not necessarily influencing the overall costs of transport (i.e. the integration of energy consumption and distance travelled per unit time, Joules/meter/second) (Lighton and Duncan, 2002; Clusella-Trullas et al., 2010).

Here we test a key climate change assumption, namely that temperate insect species are at low risk of thermal stress, by estimating thermal safety margins and warming tolerance and a range of physiological and behavioural responses to temperature variation. We further identify whether plasticity of high temperature tolerance can mediate the responses observed, and examine the effects of exposure duration on thermal limits. In addition, to further understand performance in the field and likely climate change impacts, we also assessed voluntary locomotion performance and its temperature-dependence. Finally we consider methodological influences in the measurement of habitat and insect body temperature, as different temperature measurement methods may result in dissimilar conclusions being made about species or population extinction risk.

2. Materials and methods

2.1. Study site and laboratory conditions

Ant activity and physiological tolerances were measured in the Austral summer (October/November 2009, 2010) and Austral winter (June/July 2011). *Iridomyrmex purpureus* (meat ants) were collected from a single nest on the University of New England campus, Armidale, NSW, Australia (30°29'26.02"S, 151°38'38.43"E), 980 masl. Up to 1500 worker ants were dug up at a time from the nest and placed into a 50 cm³ box with nest soil, then taken back to the laboratory. Worker ants of this species can survive for up to 12 months in captivity (Henderson et al., 2008), but were kept in captivity for 1 week to acclimate with ad libitum access to food and water. An acclimation period of 1 week was considered appropriate as other ant species have been shown to acclimate thermal tolerance traits during this time (Jumbam et al. 2008). For most insects studied to date, acclimation responses are typically asymptotic, with most traits readily altered within 3–5 days (Weldon et al. 2011). In addition, we wished to minimize any potential deleterious effects of holding animals at sub-optimal conditions and therefore balanced sufficient time for a response against a duration that might be too long. Thus, ants were kept in the laboratory for no longer than 10 days to conduct experimental procedures. Individual ants were only used once for all experimental manipulations. The following experimental methods are summarised in Table S1 (Supplementary materials and methods).

To assess the role of thermal tolerance plasticity in coping with environmental variation, we undertook seasonal sampling combined with acclimation to summer-like conditions in *I. purpureus*. Those ants used for the acclimation treatment collected in both summer (termed summer-collected summer-acclimated) and winter (termed winter-collected summer-acclimated) were housed in a constant temperature room at 25 °C for one week to acclimate

(14 h day: 10 h night photoperiod). A 25 °C summer acclimation temperature was used as a standardisation temperature which is equivalent to the mean maximum summer temperature for this location in November/December (average 25.4 °C) and 12.3 °C above the mean maximum winter temperature in June/July (12.7 °C) (BOM, 2012).

A single nest of ants (likely a polydomous nest made up of multiple, discrete nesting units; Wilgenburg et al., 2006) was studied. To account for ant genetic variability, and reduce the signal to noise ratios that occur when assessing independent genetic and physiological pathways (Santos et al., 2011), we would require at least 10 or more ant nests to be studied simultaneously: this was considered outside the scope of this study given the large sample sizes and number of traits investigated. However individual ant populations, which may be subject to different selection pressures, have been shown to respond differentially to environmental changes (Pelini et al., 2012). Nevertheless, the primary goal here – to better comprehend population extinction risk – is better addressed using the current approach, but clearly further work is required to understand population-related variation.

Photoperiod is known to influence diapause induction in ant larvae and queens (Kipiatkov and Lopatina, 2009), and egg production in ant queens and attendant workers (Hand and Smeeton 1983). Photoperiod is thought to be important for events that are

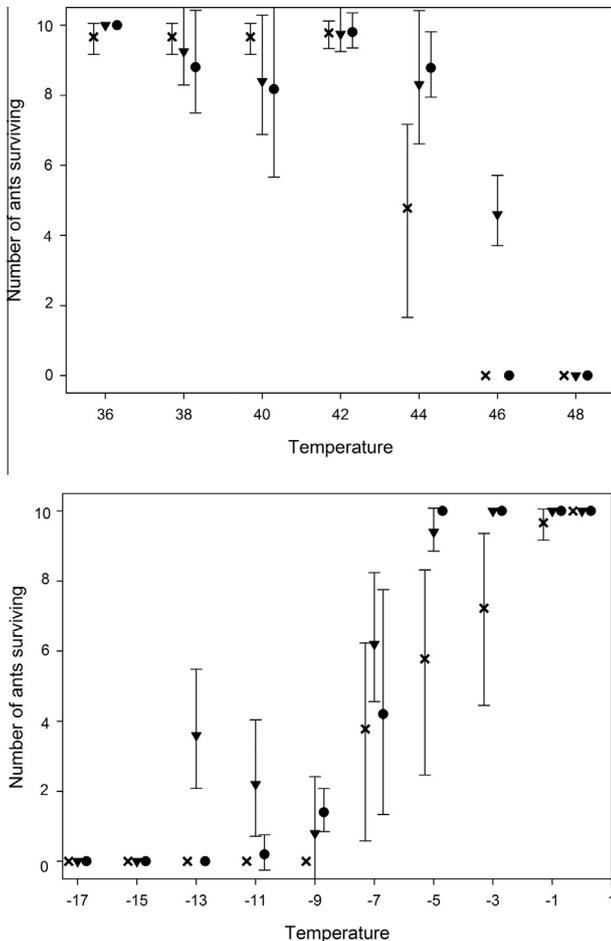


Fig. 1. Survivorship plots for ants at different test temperatures (a) upper temperatures, (b) lower temperatures from summer collected, summer acclimated, winter collected, summer acclimated or winter collected, field fresh groups. Mean survival of 10 ants in each of five replicate vials \pm s.d. shown. X – summer-collected summer-acclimated; ● – winter-collected summer-acclimated; ▼ – winter-collected field-fresh.

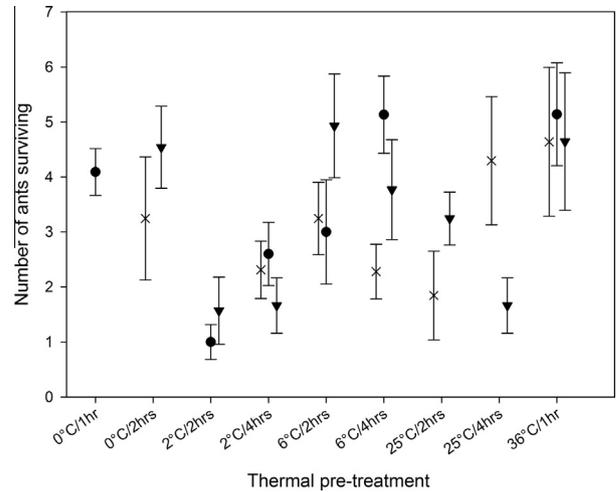


Fig. 2. Ant survival (mean survival of 10 ants in each of five replicate vials \pm s.d.) when ants were kept at the temperature resulting in 75% population mortality (-8.2 °C for 2 h) after a range of low temperature thermal pre-treatments to assess hardening (acute plasticity) in three season/acclimation groups: X – summer-collected summer-acclimated; ● – winter-collected summer-acclimated; ▼ – winter-collected field-fresh.

of a longer term (e.g. reproduction) than short term issues related to temperature or food availability (i.e. foraging; Bradshaw and Holzapfel, 2007), so it was assumed that the winter worker ants brought into the lab would not be substantially influenced by a change in photoperiod.

Samples from winter 2009 were also assessed using field fresh samples (i.e. without laboratory acclimation; labelled winter-collected field-fresh), but no field fresh samples were collected for summer-collected ants. In the first set of experiments, upper and lower lethal temperatures (ULT and LLT: high and low temperatures where 75% of a population are killed, respectively) were estimated to provide a discriminating temperature for experiments to assess acute plastic survival responses and to determine the time–temperature relationship of survival at both high and low temperatures (Powell and Bale, 2005). Upper and lower lethal temperatures were assessed by placing 50 individual workers into one of five 50 ml containers at random ($n = 10$ ants per container) and plunged directly into a Grant GP200/R4 programmable water bath at a range of static temperatures for 2 h to cover the full 0–100% mortality range (Table S1a). After 2 h, ants were removed from the water bath and returned to 25 °C in a climate controlled room for 24 h, after which they were assessed for survival (ability to right themselves after a gentle touch) (Terblanche et al., 2008). The proportion of ants surviving at each temperature was assessed using a generalized linear model (GLZ) with a binomial distribution and a logit-link function in R 2.14.1 (R Development Core Team, 2011). Since survival is a binary variable (alive or dead), we used a non-parametric generalized linear model approach (with the default logit-link function of errors given that the survival proportions are bounded at each end of the distribution), to analyze the effects of temperature on survival. This approach is similar in principal to a logistic regression (see Crawley, 2007 for background on this method and e.g. in Terblanche et al., 2008).

2.2. Acute plasticity of survival

In the second suite of experiments, we assessed a range of temperature pre-treatments to determine if exposure to sub-lethal temperatures for a range of durations might enhance ants survival

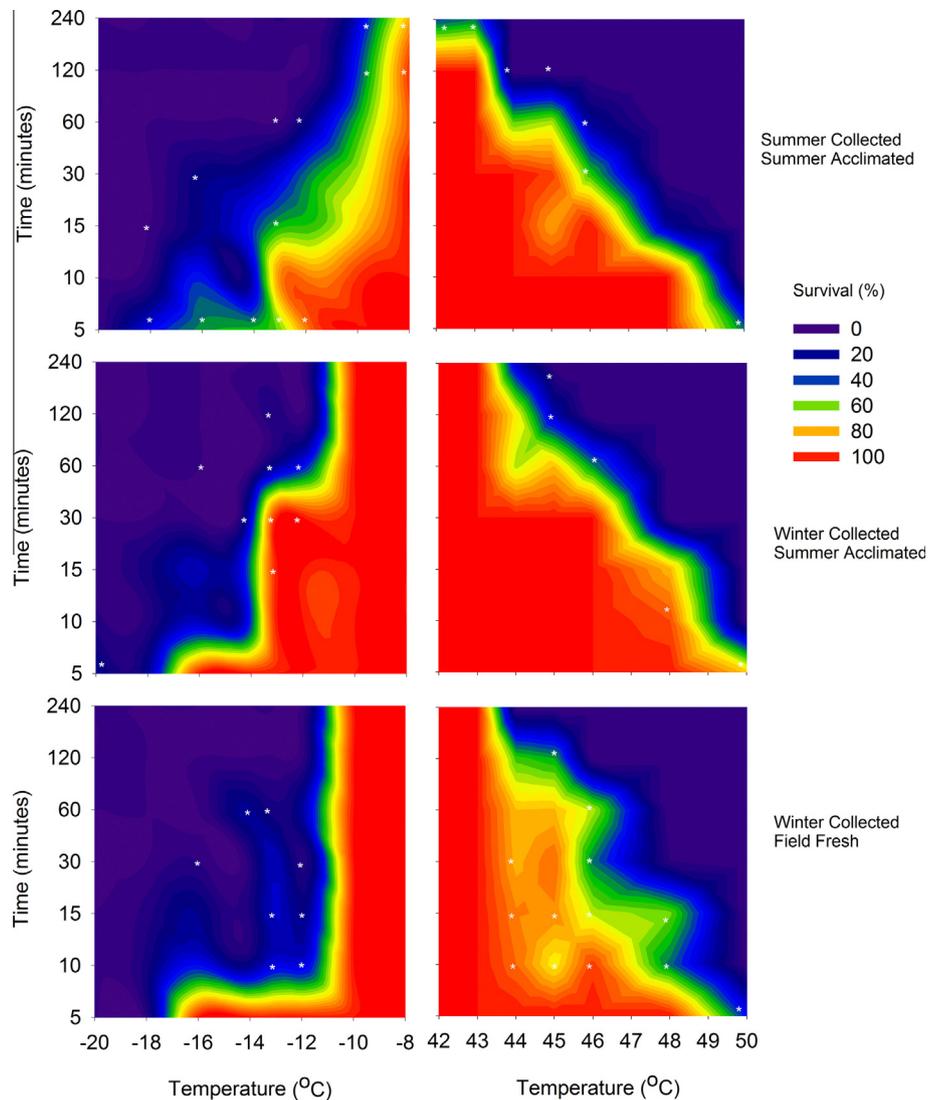


Fig. 3. Contour plots of ant survivorship (blue 0% survivorship → red 100% survivorship) at different time–temperature exposures from collections made in summer and winter. Winter samples include field fresh populations and populations acclimated for 1 week at 25 °C. Asterisks indicate significant differences ($p < 0.05$) in survival among treatments (summer collected, summer acclimated; winter collected, summer acclimated; and winter collected, field fresh).

over short time-scales (e.g. during foraging) (Table S1b). Similar to upper and lower lethal temperature assays, ants exposed to different temperature and time pre-treatments were assessed by placing 50 individuals into one of five 50 ml containers ($n = 10$ ants per vial) and plunged directly into a programmable water bath at set temperatures (at increments of 2 °C) for 2 h. Set temperature was based on the discriminating temperature, at which 25% of ants survived exposure at high or low temperature. We used 25% survival for upper and lower lethal temperatures. A 25% survival threshold was considered appropriate as it is a temperature that was lethal for most of the population, and given the rather large variability associated with these estimates. For upper and lower lethal temperatures this was 45.8 ± 1.3 °C and -8.2 ± 0.4 °C, respectively. Temperature inside vials was confirmed using a Type T copper/constantan thermocouple (Omega 36 gauge thermocouple wire: TT-T-36-SLE) connected to a Grant Squirrel SQ2020 datalogger (accuracy: 0.1 °C; verified by calibrated waterbath temperatures and NIST certified thermometers).

In order to compare upper lethal temperatures to field activity patterns, critical thermal maxima (CT_{max}) was calculated by ramping summer acclimated ants from 40 °C at 0.25 °C/min to 50 °C

(Terblanche et al., 2008; Fischer et al., 2010; Piyaphongkul et al., 2012).

2.3. Effects of duration of exposure on thermal limits

Temperature and time-dependent survivorship was assessed by placing 50 individuals into one of five 50 ml containers ($n = 10$ ants per container) and plunged directly into a programmable water bath at both upper and lower set temperatures for a range of time periods (Table S1c). After each time treatment ants were taken out of the bath and returned to 25 °C for 24 h, after which they were assessed for survival (ability to right themselves) after a gentle touch. Long-term exposure was also assessed at moderate temperatures (−1, 3, 35, and 40 °C) for 8 and 12 h (Table S1c). For each temperature and time period, the proportion of survival from the three season/acclimation treatments (summer-collected summer-acclimated, winter-collected summer-acclimated, and winter-collected field-fresh ants) was analysed separately using a generalized linear model with a binomial distribution and a logit-link function (following Terblanche et al. 2008) in R statistical software. We show only significant results ($p < 0.05$) as an asterisk mark in Fig. 3.

Table 1

Generalised Linear Model results for survival of ants for summer acclimation, winter acclimation and winter field fresh assays. Ants plunged at temperature for 2 h. Significant differences marked in bold. Na indicates no survival or full survival across all treatments and no analysis was carried out.

Temperature	DF	χ^2	<i>p</i>
-17	2	Na	Na
-15	2	Na	Na
-13	2	50.157	<0.0001
-11	2	27.594	<0.0001
-9	2	15.388	<0.0001
-7	2	7.8132	0.0201
-5	2	56.194	<0.0001
-3	2	41.612	<0.0001
-1	2	4.5368	0.1035
36	2	4.5368	0.1035
38	2	4.0957	0.129
40	2	10.456	0.0054
42	2	0.0254	0.9874
44	2	32.315	<0.0001
46	2	68.849	<0.0001
48	2	Na	Na

2.4. Activity patterns and temperature-dependent performance

Ant activity at the nest was also assessed during the sampling period (Table S1d) to identify thermal limits to foraging and peak foraging temperatures. An assessment of activity on the ant nest as well as surface temperature was taken throughout the day to maximise temperature/foraging measurements. Activity was ranked between 0 (no activity) and 3 (full activity) on a four point nominal scale (details in the online [Supplementary Information \(SI\)](#)). Full activity on the nest was considered to be when the top of the nest was covered with ants running vigorously across the nest and along adjacent, un-manipulated trails. All observers were trained in identifying different activity ranks for consistency before measurements were made. Ground surface temperature readings were taken ($\pm 1\%$ accuracy) using an infrared thermometer (Raytek Raynger ST30 Pro, USA) from a standard height above ground (1 m), 0.5 m away from the nest so as not to disturb nest ants and artificially increase ant activity. Pilot trials determined that this distance was sufficient to not alarm ants and affect behaviour. Thermal performance curves of rank activity patterns were generated using TableCurve 2D v5.01 (SYSTAT, 2002) following Angilletta (2006); details SI).

Ant running speeds in the field were measured throughout the day by recording the time ants took to run 2 m away or towards the edge of the nest along a single existing ant trail in a straight line. The primary existing ant pathway adjacent to the nest was straight and ants strictly followed this path in a straight line. Ground surface temperature readings were taken ($\pm 1\%$ accuracy) from a standard height above ground (1 m) using an infrared thermometer (Raytek Raynger ST30 Pro, USA) at the beginning, middle and end of the running trail when ants were running, and averaged for each individual ant running speed measurement. In summer, running speed of 30 ants were tested each direction (In/Out) and in winter the running speed of six ants each direction were tested. Fewer ants were available in winter due to the lower activity. As we did not physically interfere with the ants and were standing away from the ants nest and trail, we minimized any artificial running speeds caused by human interference, and assumed any interference we did cause was constant at all sampling points. Pilot trials showed that a distance of 0.5 m from the ant's trail was sufficient to avoid alarming them as long as a shadow of the observer was not cast across the trail. We attempted to minimise effects of circadian rhythm influences (Hong et al., 2007) by taking measurements

in full daylight hours across a range of days differing in weather conditions.

To assess relationships between temperature and running speed both for in situ field ants and in the laboratory acclimated ants (Table S1e) in Summer and Winter, we used model II regressions within the SMATR program (Warton et al., 2011) in R. Bivariate trait relationships were analysed by fitting Standardised Major Axis (SMA) lines to variables (temperature and running speed) to provide a better estimate of the line summarizing the relationship between two variables than a linear regression (residual variance is minimized in both *x* and *y* dimensions, rather than the *y* dimension only) (McArdle, 1988; Sokal and Rohlf, 2012). When fitting lines to the data, both linear relationships and 2nd or 3rd order polynomials had similar r^2 values (0.63–0.64), but the linear fits had lower AIC values compared to the polynomial functions and therefore only the former are reported.

To calculate topt a curve is required to work out the peak value, ideally for running speed; however running speed exhibited a linear relationship. Therefore to determine topt, and to allow subsequent calculation of thermal safety margin, we assume temperature at which activity levels are maximum is equivalent to performance optima (and see SI).

2.5. Warming tolerance and thermal safety margin

We calculated the warming tolerance and thermal safety margin for *I. purpureus* (Deutsch et al., 2008; Diamond et al., 2012). These indices were calculated not only from local weather station data (annual and summer average temperature), but also using microclimatic data collected (summer average and summer 10am–4pm average temperature), which is likely more ecologically relevant to organisms at specific microsites and useful when predicting the ecological impacts of climate change (Suggitt et al., 2011). Calculations were made for these indices from mean annual temperatures from the closest weather station, summer averages from the closest weather stations, summer soil temperatures at the ants nest (iButtons), and summer soil temperatures between 10am and 4pm on the surface (iButtons) (i.e. at peak activity times) (details provided in SI).

2.6. Comparison of surface, ant and weather station temperature readings

Finally, we tested how well microclimate temperature measurements aligned between different methods of collection. Ants were killed using standard protocol for insects of ethyl acetate for 15 min. Measuring temperatures of deceased ants can cause confounding effects due to desiccation and evaporative water loss reducing body temperature. We therefore kept the body temperature recordings to a 3 h period only. We compared the infrared thermometer obtained estimates to ground-exposed thermocouples, and also thermocouples with freshly deceased ants attached (both using Omega 40 gauge Type T Copper-Constantan thermocouple wire (TT-T-40-SLE) connected to a Pacific Data Systems DT80 Temperature Monitoring Station) and to the closest weather station (UNE Kirby – approximately 2 km from the study site). Measurements were only taken over a 3 h period to reduce the effects of ant desiccation over time, as the ants were freshly deceased before the thermocouples were attached via a small drop of fast drying adhesive glue and the thermocouple tip was placed against the ventral thorax surface. Ants were then point-mounted and placed on a pin so they were 2–3 mm above the surface of the ground to mimic the natural distance from the ground when ants are running. Regressions were calculated in Sigmaplot 12.5 to compare the four different temperature reading methods.

Table 2

Acute plasticity of survival results for ants collection from within each of the three season/acclimation groups, and pre-treated at different temperatures and times. Full range of temperatures and pre-treatments shown in Table S1b. Only significant differences shown ($p > 0.05$).

Season/acclimation	Pretreatments	df	χ^2	p
Winter-collected Field-fresh	0 °C 2 h vs 2 °C 2 h	1	8.3787	0.0038
	2 °C 2 h vs 2 °C 4 h	1	30.521	<0.0001
	2 °C 4 h vs 6 °C 4 h	1	4.2556	0.0391
Winter-collected Summer-acclimated	2 °C 2 h vs 6 °C 2 h	1	6.4858	0.0109
	2 °C 4 h vs 6 °C 4 h	1	6.6369	0.0099
	6 °C 4 h vs 6 °C 4 h	1	10.674	0.0011
	36 °C 1 h vs 36 °C 1 h	1	4.1664	0.0412
Summer-collected Summer-acclimated	25 °C 2 h vs 25 °C 4 h	1	40.801	<0.0001

3. Results

3.1. Lethal limits

Ant survivorship across the high and low temperature lethal spectrum was dependent on season (Fig. 1). Winter-collected field-fresh ants had significantly higher survival than summer-collected summer-acclimated or winter-collected summer-acclimated ants at -13, -11, and -7 °C. By contrast, at -9, -5 and -3 °C summer-collected lab-acclimated ants had significantly lower survival compared to other treatments (Fig. 1; Table 1). At high temperatures, summer-collected lab-acclimated ants had signifi-

Table 3

SMATR analysis of ant running speeds in the field. (a) Test for common slope among treatments, (b) Standardised Major Axis' results, (c) Post-hoc multiple comparisons of slopes among treatments (p values only). Significant values in bold.

(a) Test for common slope among treatments				
df	D	p		
3	12.04	0.0007		
(b) SMA Results				
Treatment	Code	n	r^2	p
Summer In	SI	30	0.633	<0.0001
Summer Out	SO	30	0.732	<0.0001
Winter In	WI	6	0.000	0.933
Winter Out	WO	6	0.175	0.410
(c) Post-hoc multiple comparison p values of slopes among treatments				
Group	SI	SO	WI	
SO	0.002			
WI	0.398	0.867		
WO	0.928	0.266	0.505	

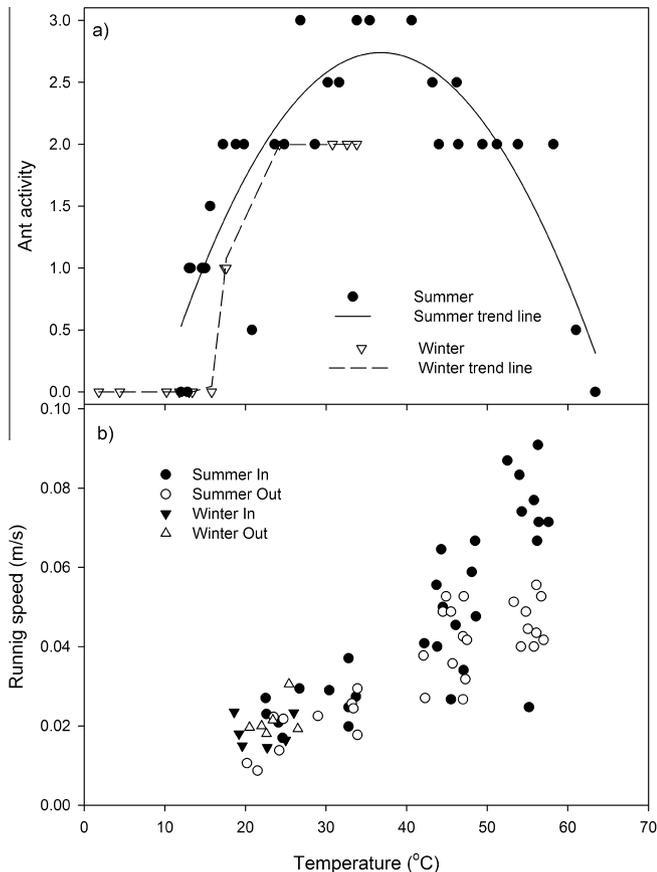


Fig. 4. (a) Ant activity at different temperatures in summer and winter. Summer trend lines also included. Ant activity axis: 0 = no activity to 3 = full activity. Trend lines of best fit are also shown: in summer it was a modified Gaussian curve (Table S2a) and in winter, it was a linear standard relationship with y-transformed single term without an intercept (Table S2b); (b) ant field running speeds at different temperatures in winter and summer towards the nest (In) and away from the nest (Out).

cantly greater heat survival at 40 °C, but survived significantly poorer at 44 °C relative to the other groups. Surprisingly, at 46 °C winter-collected field-fresh ants had significantly higher survival than summer-collected lab-acclimated or winter-collected lab-acclimated ants (Fig. 1; Table 1).

3.2. Acute plasticity of lethal limits

Temperature pre-treatment did not result in improved high temperature survival with few ants surviving ($0.6 \pm 5.6\%$ ants surviving across all pre-treatments). Ant survival when exposed to the lower lethal temperature of -8.2 °C for 2 h varied depending on the exact temperature pre-treatments at the different temperature and time combinations (Fig. 1). Comparison among seasons within a particular time-temperature treatment revealed substantial variability in survival. For example, no differences in low temperature survival across groups could be detected with a 36 °C for 1 h treatment and also in the 2 °C for 4 h treatment. By contrast, summer-collected summer-acclimated ants fared more poorly after 4 h at 6 °C than winter-collected field-fresh or winter-collected summer-acclimated. Similarly, winter-collected, field-fresh ants survived better at 6 °C for 2 h compared to winter-collected summer-acclimated or summer-collected summer-acclimated ants exposed to the same conditions (Fig. 2 and Table 2).

When summer-collected summer-acclimated, winter-collected summer-acclimated, and winter-collected, field-fresh ants were exposed to different temperatures for different time periods, survival varied significantly (Fig. 3: significant differences, $p < 0.05$, identified by an asterisk). At -12 °C for 10, 15 and 30 min, winter-collected field-fresh samples had lower survival compared to the two acclimated treatments. At -10 and -8 °C, summer-collected, summer-acclimated samples had a lower survival

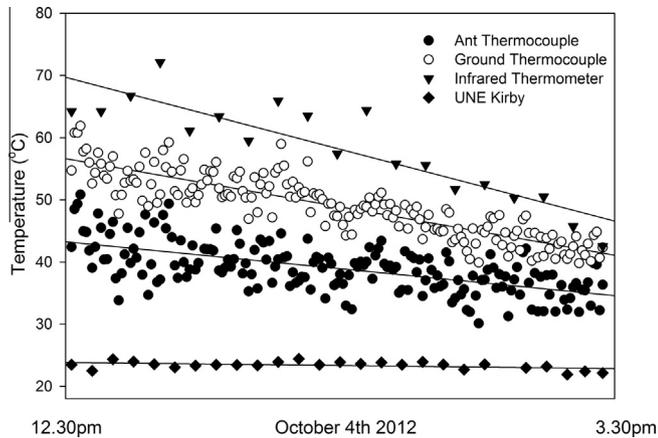


Fig. 5. Comparison of microclimate temperature measurements from the local weather station (UNE Kirby 30°43.87'S, 151°62.93'E), and local surface temperatures at UNE using an infrared thermometer, exposed thermocouple wire to the ground, and thermocouple attached directly to the exoskeleton of an ant (2–3 mm above ground surface) over 3 h in October 2012.

compared to the winter-collected, summer-acclimated treatments at 120 and 240 min exposure. For higher temperatures, summer-collected summer-acclimated ants had lower survival when exposed to 42 and 43 °C for 240 min compared to the winter-collected summer-acclimated treatments. Winter-collected, field-fresh ants had a lower survival at higher temperatures (44C, 45, 46 and 48 °C) compared to the two summer acclimated treatments for 10, 15 and 30 min.

Critical thermal maxima of ants was determined at 46.1 °C (± 0.1 °C; $n = 11$) under the measurement conditions used here. Ants were active until 44.1 °C when they became unstable on their tarsi. At 46.6 °C they would attempt to run and then fall over consistently. By 47.2 °C they were unable to right themselves after falling over, and by 48.4 °C they exhibited no movement at all.

3.3. Ant field activity levels

Nest activity showed different responses in summer and winter. In summer, ant activity on the nest started when the surface temperature was 12.9 °C and stopped when the surface temperature increased above 63 °C (Fig. 4a). Highest levels of activity occurred between 25 and 42 °C. The best thermal performance curve to explain ant activity in summer was a modified Gaussian curve (Table S2a). Ant activity was more lethargic in winter, and started at 17 °C. Activity did not exceed a ranking of 2 when the temperature reached 24.2 °C and was still ranked 2 at the warmest winter surface temperature recorded at 33.8 °C. In winter the best thermal performance curve to explain ant activity was a linear standard relationship with y -transformed single term without an intercept (Table S2b).

Table 4

Warming tolerance and thermal safety margin calculations (following Deutsch et al. 2008) for *Iridomyrmex purpureus* based on different T_{hab} sources (local weather station – annual average, summer average; iButton data loggers at ants nest – summer average, summer 10am–4pm average).

Temperature source for T_{hab}	Warming tolerance (°C)	Thermal safety margin (°C)
Weather station annual average	25.8	13.78
Weather station summer average	19.52	7.51
iButton summer average	19.12	7.11
iButton summer 10am–4pm average	7.81	–4.2

T_{hab} is the calculation of the average temperature in the ants habitat.

T_{opt} was calculated on the average temperature of highest ant activity (Fig. 3a). No optimum could be derived from running performance.

Warming tolerance = $CT_{max} - T_{hab}$.

Thermal safety margin = $T_{opt} - T_{hab}$.

3.4. Temperature-dependent running speeds

The voluntary running speeds of ants varied significantly (Fig. 4b; Table 3a: test for common slope among treatments) with a significant increase in running speed as temperature increased for both in situ summer-in and summer-out, but no temperature/time relationship for the samples taken in winter (Fig. 4b; Table 1b: SMA Results). Ants running towards the nest in summer (summer-in) had significantly higher running speeds than those leaving the nest in summer (summer-out) (Fig. 4b, Table 3c). The soil surface temperatures that ants exhibited voluntary running ability ranged between 18.6 and 57 °C. Based on the temperature relationships developed in Fig. 5 this equates to a body temperature of approximately 24.5–43.1 °C.

3.5. Nest temperature

In summer, average temperatures at 0.5 cm below the surface of the ant's nest ranged between 9 °C and 56 °C, with an average (\pm s.d.) of 27.0 (± 10.6)°C (Fig. S1a). At 15 cm depth into the ant's nest, average summer temperatures ranged between 11 °C and 46 °C, with an average of 26.5 \pm 7.3 °C (Fig. S1b). In winter, average temperatures at 0.5 cm below the surface of the ant's nest ranged between –5.5 °C and 26.5 °C, with an average of 6.1 \pm 5.6 °C (Fig. S1c). At 15 cm into the ants nest average winter temperatures ranged between 0.5 °C and 22.5 °C, with an average of 6.4 \pm 2.9 °C (Fig. S1d). Nest temperatures over 50 °C were found only in summer on top of the nest (i.e. on the trail surface). Temperatures exceeded 50 °C seven times during the summer months, five times for 4 h, once for 2 h and once for 1 h duration.

3.6. Warming tolerance and thermal safety margins

Warming tolerance and thermal safety margin were estimated as 25.8 °C and 13.8 °C, respectively at the Armidale site (latitude: 30.52°S) when using mean annual temperatures (Table 4). Warming tolerance and thermal safety margin reduced systematically as temperature readings became more site-specific: from weather station annual average through to the iButton summer 10am–4pm average (Table 4).

3.7. Comparison of surface, ant and weather station temperature readings

Temperature measurements using different methods were compared over a three hour period (Fig. 5). The infrared thermometer had the highest temperature estimates ($y = 69.57 - 0.12x$, $r^2 = 0.78$), consistently higher temperatures than ground-exposed thermocouples ($y = 43.21 - 0.05x$, $r^2 = 0.38$) and body temperatures estimated for ants attached to thermocouples ($y = 56.54 - 0.08x$, $r^2 = 0.19$). Air temperatures from the closest weather station were at least 10 °C lower than temperatures on the ground ($y = 23.85 - 0.01x$, $r^2 = 0.19$).

4. Discussion

4.1. Ant survival and thermal stress

Assessing physiological responses of organisms to climate change is crucial (Williams et al., 2008) and is surprisingly not a major focal area of research examining insect responses to climate change (6% of papers studied assessed insect physiological responses to both simulated and realised climate change; Andrew et al., 2013). Moreover, it is critical to understand changes to populations of common species (Gaston, 2011) as changes in population structure may lead to the collapse in trophic interactions and ecosystem services (Andrew, 2013). It is anticipated that, because they are abundant and live within temperate environments, they are relatively more resilient to rapid environmental change compared to organisms in tropical habitats (Deutsch et al., 2008; Diamond et al., 2012). *I. purpureus* tested in this study exhibited a similar upper lethal temperature (45.8 °C) and CT_{max} (46.1 °C) to 11 species of ants (CT_{max} range 40–50 °C and upper lethal temperatures range 42–56 °C) collected from Barcelona, Spain (Cerdeira et al., 1998). Upper lethal temperatures and CT_{max} are, however, fundamentally different measures of tolerance to thermal stress (see e.g. recent review in Terblanche et al., 2011). Ant body and ground surface temperatures (whether measured with an infrared thermometer or thermocouple) show a much higher temperature in the middle of the day than air temperatures measured at the same time (Fig. 5). With climate change, mean air temperatures are expected to increase between 2 and 6 °C over the coming century (IPCC, 2007), which would suggest much higher temperatures on exposed ground in which *I. purpureus* carry out most of their above-ground activities. This suggests that these ants will be exposed to temperatures above their CT_{max} for a longer period of time when above ground. Even in spring (October) in Australia, ant body temperatures increase above the CT_{max} estimates, and yet they still remain active. Understanding how close these species' thermal limits are to the maximum surface and expected ant body temperatures within their environments is crucial to assess when and for how much time these populations may be exposed to extreme thermal stress, or to understand their use of behavioural thermoregulation to avoid injury and maintain function (Kearney et al., 2009).

Given the range of results reported here which attempted to induce variation in high temperature tolerance, plasticity of thermal tolerance is unlikely to be aiding this population of *I. purpureus* and thereby improving their high temperature tolerance in summer or winter (Fig. 3). Furthermore, there is little evidence for a heat hardening response across the range of conditions examined (see review in e.g. Hoffmann et al., 2003; Chown and Nicolson, 2004). Indeed, we found that pre-treating ants at a range of temperatures did not improve their survival at a high discriminating temperature but it did improve survival at a low lethal temperature (although not necessarily in a consistent manner). The major thermal plasticity responses of this species are at the lower temperatures where summer acclimation increases low temperature survival. This is similar to many other insect species (e.g. Fischer et al., 2010; Lachenicht et al., 2010) and other ant species (Jumbam et al., 2008) and supports the general idea that upper and lower tolerance is decoupled in insects (Chown and Nicolson, 2004; Addo-Bediako et al., 2000).

4.2. Ant activity

We found *I. purpureus* reduces its activity at higher temperatures (above 50 °C, soil surface temperature) compared to

moderate temperatures (25–40 °C; Fig. 4a), but their performance based on running speed estimates in the field does not (Fig. 4b). In fact running speed exhibits a linear increase with temperature rather than the expected, non-linear 'thermal performance curve' reduction (Angilletta et al., 2002) at the highest recorded summer surface temperatures (56 °C). Running speeds are expected to decrease at surface temperatures above 50 °C due to the physiological stress that such high temperatures would place on the ants. The lack of an expected thermal performance curve indicates that the ants may be using behavioural cues to deal with the extreme temperatures (Anderson et al., 1979; Lactin and Johnson, 1998; Kearney et al., 2009). Such behavioural thermoregulation cues could include not leaving the nest at all, running faster back to the nest, raising their gaster or climbing grass or other three-dimensional objects to obtain access to cooler air above ground layer, changing foraging routes to spend more time in cooler microclimates (e.g. shade), or feeding on foraging resources to overcome heat stress and taking smaller loads back to the nest. When these ants are foraging in extreme heat conditions they can still perform at a high running capacity with no sign of speed reduction. This result calls into question the general application of thermal performance curves to predict likely extinction risk, (e.g. Deutsch et al., 2008; see also discussion in Kearney et al., 2009) without taking into account behavioural flexibility.

The direct measures of the trail temperature are almost in equilibrium with body temperature of ectothermic organisms that are small in size (Porter and Gates, 1969; Helmuth et al., 2005). From a biophysical point of view this can be important as exchange of heat, mass, and momentum between organisms and their environment explicitly links organisms with their environment at the physiological and biomolecular scale (Bakken, 1976; Casey, 1988; Helmuth et al., 2005). We did not assess the individual body temperature of each ant, but the environmental temperature measurements taken are likely a good approximation of the potential thermal stress experienced. Here we tested different temperature measurement methods (Fig. 5) and found that even though the infrared thermometer overestimates ant body temperatures, so did the use of exposed thermocouples. However, and as might be largely expected from the physics of heat exchange (e.g. Gates, 1980; Campbell and Norman, 1998), both of these methods were more consistent with body temperature estimates made with a thermocouple attached to the ant directly than the use of the closest weather station (2 km away).

4.3. Influence of microclimate and thermal calculations

Assessments such as this one exemplify the need to integrate local microclimatic conditions into predictable response calculations, such as warming tolerance and thermal safety margin, not just annual climatic averages from large-scale databases (Suggitt et al., 2011) or even air temperature (Helmuth et al., 2010). Furthermore, our results suggest that soil-dwelling taxa in temperate ecosystems may also be highly vulnerable to climate change, especially highly active species that voluntarily forage in exposed areas at the edge of their thermal tolerance, particularly in summer. However, this vulnerability needs to take into account behavioural adaptations and their flexibility (such as stopping above-ground foraging when the temperatures are extreme). Our results further suggest that the behavioural abilities of insect species also need to be incorporated into a theoretical framework in order to predict extinction risk with climate change. If the pattern we observe is more broadly applicable to other sites and insect species, one potential implication is that terrestrial insect species are at lower risk than currently implied. However, there may well be cases of

species for which their climate change-related extinction risk is underestimated, for example, less mobile life-stages or species.

Although activity appears to be undertaken at temperatures greater than thermal limits or activity limits estimated in the laboratory, this is likely explained by the overestimation of body temperature by simply measuring trail temperatures using an IR thermometer. Indeed, IR integrates temperature across a relatively large surface area (75 mm wide area when pointed at surface from 900 mm away) and ignores surface rugosity and trail soil surface complexity issues. In this study, ants were much more active at warmer temperatures, with their activity levels being greatest at ground temperatures which are similar to their upper lethal temperature estimates. Perhaps somewhat surprisingly, some ants were still active and outside of the nest at trail temperatures of 61 °C (measured using an infrared thermometer). However, from our temperature measurement trials (Fig. 5) a trail temperature of 61 °C estimated from the IR thermometer would be equivalent to an ant body temperature of *c* 42 °C, and is in agreement with body temperature predicted from running speed in the laboratory (results not shown).

A critical issue in assessing thermal limits and potential extinction risk is variation among methods and its relevance to field rates of temperature change (reviewed in e.g. Helmuth et al., 2005; Terblanche et al., 2011). Other factors that influence these measurements are a host of extrinsic and intrinsic factors, such as changing body condition (e.g. thermal or hygric stress, Overgaard et al., 2012), method of assessment and the voluntary or involuntary nature of the trait itself (Hazell and Bale, 2011) and thermal history at various time-scales (Fischer et al., 2010; Jumbam et al., 2008) (Fig. 3).

4.4. Warming tolerance and thermal safety margins

Warming tolerance and thermal safety margins for *I. purpureus* were at the higher margin (for TSM), or higher (WT) than those found by Deutsch et al. (2008) based on calculations using mean annual temperature. However, when summer average temperatures and site-specific, nest-top temperature was used, the warming tolerance and thermal safety margin were equivalent or substantially lower than those found by Deutsch et al. (2008). In addition, the expected warming tolerance of ants from Diamond et al.'s (2012) predictions for ants at -30° latitude is $\sim 15^{\circ}\text{C}$ which was generated from CT_{max} data derived from existing literature and temperature data from WorldClim global database built from typical weather stations (Hijmans et al., 2005). Warming tolerance of *I. purpureus* is higher when compared using the equivalent weather station annual average calculation for our data (25.8 °C). However, when based on ecologically relevant microclimate data (Suggitt et al., 2011) using the microclimate temperatures at the hottest time of the day (10am–4pm) to calculate warming tolerance and thermal safety margin (7.81 and -4.2°C , respectively), we found that the ants foraging during summer are voluntarily exposing themselves to extreme thermal soil conditions. This could explain why meat ants are much faster at returning to their nest (Fig. 4b) than leaving it. Running faster back to the nest could be for two reasons which need further work to disentangle: firstly, ants may be more motivated and need heat respite from potential damage since thermal refugia (e.g. grass blades) are not present on their trails; and secondly, they are warmer and therefore faster, but not necessarily damaged. Further work is needed to distinguish among these hypotheses. Negative estimates of thermal safety margins are also found in squamate ectotherms (lizards and snakes) at 30° latitude when thermal safety margin are calculated using mean temperature of the warmest quarter (Clusella-Trullas et al., 2011).

5. Conclusions

We have shown, at least for our temperate meat ant population, that they are voluntarily exposing themselves, or active, close to their thermal maxima, and these upper limits have limited flexibility within a single generation or even between seasons. Furthermore, given results from other insects examined to date (e.g. Kellermann et al., 2012) they may generally be constrained from an evolutionary perspective (see also Hoffmann et al., 2012). For ants, this means the colony may have to employ a range of strategies to overcome temperature stress which may include: adapt to having a larger number of workers killed from heat stress making them prone to local extinction; start foraging at different times of the day to avoid extreme temperatures; develop strategic behaviours to reduce exposure to extreme temperatures when out foraging, such as climbing grass blades to cool down or foraging only within shaded areas. Although these results only incorporated ants from a single nest, the outcomes are nevertheless insightful. Further work could expand these results to repeated observations on multiple nests, but was considered outside of the scope of the present study owing to logistic constraints. The results are critical for the local population but whether these results hold for other populations or ant species more generally requires further work, as there are known differences in physiology among populations of ants; e.g. *Myrmecia* spp. (Elmes et al., 1999; Nielsen et al., 1999) and *Atta texana* (Mueller et al., 2011). Understanding inter-population variation in key traits and gene flow among populations are critical to estimates of adaptive evolutionary potential, although high temperature tolerance appears generally constrained (Hoffmann et al., 2012; Kellermann et al., 2012). Common insects, such as meat ants, will likely be exposed to higher thermal extremes during summer (AAS, 2010; CSIRO-ABM, 2012), so their thermal safety margin will continue to be reduced, potentially exposing them to even more heat stress if they maintain the same behaviours in the future. Other factors such photoperiod (Bradshaw and Holzapfel, 2010; Bradshaw and Holzapfel, 2007), solar elevation (Amor et al., 2011) and/or interspecific competition (Albrecht and Gotelli, 2001) may also constrain behavioural flexibility to deal with extreme temperature exposure. Adaptive capacity to a changing climate, including evolutionary constraint of adaptive capacity (e.g. Kellermann et al., 2012) and plastic ecological responses also need to be considered (Williams et al., 2008; Hoffmann et al., 2003). In conclusion, the relative costs and benefits for local population persistence of, and behavioural modification to, this predicted climate-change related warming should be carefully considered before attempting to predict the fate of the species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jinsphys.2013.06.003>.

References

- AAS, 2010. The Science of Climate Change: Questions and Answers. Australian Academy of Science. Available at: <<http://www.science.org.au/reports/climatechange2010.pdf>>.
- Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society B: Biological Sciences* 267, 739–745.
- Albrecht, M., Gotelli, N.J., 2001. Spatial and temporal niche partitioning in grassland ants. *Oecologia* 126, 134–141.
- Amor, F., Ortega, P., Cerdá, X., Boulay, R.R., 2011. Solar elevation triggers foraging activity in a thermophilic ant. *Ethology* 117, 1031–1039.
- Andersen, A.N., 2000. The Ants of Northern Australia: A Guide to the Momsoonal Fauna. CSIRO, Collingwood.
- Anderson, R.V., Tracy, C.R., Abramsky, Z., 1979. Habitat selection in two species of short-horned grasshoppers. *Oecologia* 38, 359–374.
- Andrew, N.R., 2013. Population dynamics of insect populations: impacts of a changing climate. In: Rohde, K. (Ed.), *The Balance of Nature and Climate Change*, Cambridge University Press, pp. 311–324.
- Andrew, N.R., Terblanche, J.S., 2013. Insects. In: Salinger, J. (Ed.), *Climate of Change: Living in a Warmer World*. David Bateman Ltd., Auckland, pp. 38–50.
- Andrew, N.R., Hill, S.J., Binns, M., Bahar, M.H., Ridley, E.V., Jung, M.-P., Fyfe, C., Yates, M., Khusro, M., 2013. Assessing insect responses to climate change: what are we testing for? Where should we be heading? *PeerJ* 1, e11.
- Angilletta, M.J., 2006. Estimating and comparing thermal performance curves. *Journal of Thermal Biology* 31, 541–545.
- Angilletta, M.J., 2009. *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, New York.
- Angilletta, M.J., Niewiarowski, P.H., Navas, C.A., 2002. The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* 27, 249–268.
- Bakken, G.S., 1976. A heat transfer analysis of animals: unifying concepts and the application of metabolism chamber data to field ecology. *Journal of Theoretical Biology* 60, 337–384.
- BOM, 2012. Climate Statistics for Australian Locations: Summary Statistics Armidale (Radio Station 2AD). Australian Government Bureau of Meteorology. Available at: <http://www.bom.gov.au/climate/averages/tables/cw_056002.shtml>. (Data extracted 12.01.12).
- Bonebrake, T.C., Deutsch, C.A., 2012. Climate heterogeneity modulates impact of warming on tropical insects. *Ecology* 93, 449–455.
- Bradshaw, W.E., Holzapfel, C.M., 2007. Evolution of animal photoperiodism. *Annual Review of Ecology, Evolution, and Systematics* 38, 1–25.
- Bradshaw, W.E., Holzapfel, C.M., 2010. Light, time, and the physiology of biotic response to rapid climate change in animals. *Annual Review of Physiology* 72, 147–166.
- Calosi, P., Bilton, D.T., Spicer, J.I., 2008. Thermal tolerance, acclimatory capacity and vulnerability to global climate change. *Biology Letters* 4, 99–102.
- Campbell, G.S., Norman, J.M., 1998. *An Introduction to Environmental Biophysics*. Springer-Verlag, New York.
- CAS, (2012). Antweb. The California Academy of Sciences. Version 4.68. Available at: <<http://www.antweb.org/allantweb.jsp>>.
- Casey, T.M., (1988). Thermoregulation and heat exchange. In: Evans, P.D., Wigglesworth, V.B. (Eds.), *Advances in Insect Physiology*, vol. 20. Academic Press, pp. 119–146.
- Cerda, X., Retana, J., Cros, S., 1998. Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. *Functional Ecology* 12, 45–55.
- Chown, S.L., Nicolson, S.W., 2004. *Insect Physiological Ecology: Mechanisms and Patterns*. Oxford University Press, Oxford.
- Chown, S.L., Hoffmann, A.A., Kristensen, T.N., Angilletta, M.J., Stenseth, N.C., Pertoldi, C., 2010. Adapting to climate change: a perspective from evolutionary physiology. *Climate Research* 43, 3–15.
- Christian, K.A., Morton, S.R., 1992. Extreme thermophilia in a central Australian ant, *Melophorus bagoti*. *Physiological Zoology* 65, 885–905.
- Clusella-Trullas, S., Chown, S.L., 2011. Comment on “Erosion of lizard diversity by climate change and altered thermal niches”. *Science* 332, 537.
- Clusella-Trullas, S., Terblanche, J.S., Chown, S.L., 2010. Phenotypic plasticity of locomotion performance in the seed harvester *Messor capensis* (Formicidae). *Physiological and Biochemical Zoology* 83, 519–530.
- Clusella-Trullas, S., Blackburn, T.M., Chown, S.L., 2011. Climatic predictors of temperature performance curve parameters in ectotherms imply complex responses to climate change. *The American Naturalist* 177, 738–751.
- Crawley, M.J., 2007. *The R Book*. John Wiley and Sons, Chichester.
- CSIRO, (2007). *Climate Change in Australia: Observed Changes and Projections*. CSIRO, Canberra. Available at: <http://www.climatechangeinaustralia.gov.au>.
- CSIRO-ABM, (2012). State of the Climate 2012. CSIRO and the Australian Bureau of Meteorology, Canberra. Available at: <<http://www.csiro.au/Outcomes/Climate/Understanding/State-of-the-Climite-2012.aspx>>.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C., Martin, P.R., 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America* 105, 6668–6672.
- Diamond, S.E., Magdalena Sorger, D., Hulcr, J., Pelini, S.L., Toro, I.D., Hirsch, C., Oberg, E., Dunn, R.R., 2012. Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Global Change Biology* 18, 448–456.
- Elmes, G.W., Wardlaw, J.C., Nielsen, M.G., Kipyatkov, V., Lopatina, E.B., Radchenko, A.G., Barr, B., 1999. Site latitude influences on respiration rate, fat content and the ability of worker ants to rear larvae: a comparison of *Myrmica rubra* (Hymenoptera: Formicidae) populations over their European range. *European Journal of Entomology* 96, 117–124.
- Fischer, K., Dierks, A., Franke, K., Geister, T.L., Liszka, M., Winter, S., Pflücke, C., 2010. Environmental effects on temperature stress resistance in the tropical butterfly *Bicyclus anynana*. *PLoS ONE* 5, e15284.
- Gaston, K.J., 2011. Common ecology. *BioScience* 61, 354–362.
- Gates, D.M., 1980. *Biophysical Ecology*. Spinger-Verlag, New York.
- Greenaway, P., 1981. Temperature limits to trailing activity in the Australian arid-zone meat ant *Iridomyrmex purpureus* form *viridiaeneus*. *Australian Journal of Zoology* 29, 621–630.
- Hansen, J., Sato, M., Ruedy, R., 2012. Perception of climate change. *Proceedings of the National Academy of Sciences of the United States of America* 109, 14726–14727 (online early).
- Hand, L., Smeeton, N., 1983. The effect of photoperiod by the red ant, *Myrmica rubra*. *Entomologia Experimentalis et Applicata* 34, 169–173.
- Hazell, S.P., Bale, J.S., 2011. Low temperature thresholds: Are chill coma and CTmin synonymous? *Journal of Insect Physiology* 57, 1085–1089.
- Helmuth, B., Kingsolver, J.G., Carrington, E., 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? *Annual Review of Physiology* 67, 177–201.
- Helmuth, B., Broitman, B.R., Yamane, L., Gilman, S.E., Mach, K., Mislan, K.A.S., Denny, M.W., 2010. Organismal climatology: analyzing environmental variability at scales relevant to physiological stress. *Journal of Experimental Biology* 213, 995–1003.
- Henderson, A., Henderson, D., Sinclair, J., 2008. *Bugs Alive: A Guide to Keeping Australian Invertebrates*. Museum Victoria, Melbourne.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25, 1965–1978.
- Hoffmann, A.A., Sørensen, J.G., Loeschcke, V., 2003. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *Journal of Thermal Biology* 28, 175–216.
- Hoffmann, A.A., Chown, S.L., Clusella-Trullas, S., 2012. Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology* (online early).
- Hong, C.I., Conrad, E.D., Tyson, J.J., 2007. A proposal for robust temperature compensation of circadian rhythms. *Proceedings of the National Academy of Sciences of the United States of America* 104, 1195–1200.
- Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Pérez, H.J.A., Garland Jr., T., 2009. Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B: Biological Sciences* 276, 1939–1948.
- Hurlbert, A.H., Ballantyne, F., Powell, S., 2008. Shaking a leg and hot to trot: the effects of body size and temperature on running speed in ants. *Ecological Entomology* 33, 144–154.
- IPCC, 2007. Summary for Policymakers. In *Climate Change 2007: The Physical Science Basis*. In: Solomon, S., Qin, D., Manning, M., et al. (Eds.), *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, pp. 1–18.
- Jayatilaka, P., Narendra, A., Reid, S.F., Cooper, P., Zeil, J., 2011. Different effects of temperature on foraging activity schedules in sympatric *Myrmecia* ants. *The Journal of Experimental Biology* 214, 2730–2738.
- Jumbam, K.R., Jackson, S., Terblanche, J.S., McGeoch, M.A., Chown, S.L., 2008. Acclimation effects on critical and lethal thermal limits of workers of the Argentine ant, *Linepithema humile*. *Journal of Insect Physiology* 54, 1008–1014.
- Kearney, M., Shine, R., Porter, W.P., 2009. The potential for behavioral thermoregulation to buffer “cold-blooded” animals against climate warming. *Proceedings of the National Academy of Sciences of the United States of America* 106, 3835–3840.
- Kellermann, V., Overgaard, J., Hoffmann, A.A., Fløjgaard, C., Svenning, J.-C., Loeschcke, V., 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proceedings of the National Academy of Sciences of the United States of America* 109, 16228–16233.
- Kipiatkov, V.E., Lopatina, E.B., 2009. Temperature and photoperiodic control of diapause induction in the ant *Lepisiota semenovi* (Hymenoptera, Formicidae) from Turkmenistan. *Journal of Evolutionary Biochemistry and Physiology* 45, 238–245.
- Lach, L., Parr, C.L., Abbott, K.L., 2010. *Ant Ecology*. Oxford University Press, New York.
- Lachenicht, M.W., Clusella-Trullas, S., Boardman, L., Le Roux, C., Terblanche, J.S., 2010. Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). *Journal of Insect Physiology* 56, 822–830.
- Lactin, D.J., Johnson, D.L., 1998. Convective heat loss and change in body temperature of grasshopper and locust nymphs: relative importance of wind speed, insect size and insect orientation. *Journal of Thermal Biology* 23, 5–13.
- Lighton, J.R.B., Duncan, F.D., 2002. Energy cost of locomotion: validation of laboratory data by in situ respirometry. *Ecology* 83, 3517–3522.
- Maysov, A., Kipyatkov, V.E., 2009. Critical thermal minima, their spatial and temporal variation and response to hardening in *Myrmica* ants. *Cryo-Letters* 30, 29–40.
- McArdle, B.H., 1988. The structural relationship – regression in biology. *Canadian Journal of Zoology* 66, 2329–2339.

- Mueller, U.G., Mikheyev, A.S., Hong, E., Sen, R., Warren, D.L., Solomon, S.E., Ishak, H.D., Cooper, M., Miller, J.L., Shaffer, K.A., Juenger, T.E., 2011. Evolution of cold-tolerant fungal symbionts permits winter fungiculture by leafcutter ants at the northern frontier of a tropical ant–fungus symbiosis. *Proceedings of the National Academy of Sciences of the United States of America* (online early).
- Nielsen, M.G., Elmes, G.W., Kipyatkov, V.E., 1999. Respiratory Q10 varies between populations of two species of *Myrmica* ants according to the latitude of their sites. *Journal of Insect Physiology* 45, 559–564.
- Nyamukondiwa, C., Terblanche, J.S., Marshall, K.E., Sinclair, B.J., 2011. Basal cold but not heat tolerance constrains plasticity among *Drosophila* species (Diptera: Drosophilidae). *Journal of Evolutionary Biology* 24, 1927–1938.
- Oberg, E., Del Toro, I., Pelini, S., 2012. Characterization of the thermal tolerances of forest ants of New England. *Insectes Sociaux* 59, 167–174.
- Overgaard, J., Kristensen, T.N., Mitchell, K.A., Hoffmann, A.A., 2011. Thermal tolerance in widespread and tropical *Drosophila* species: does phenotypic plasticity increase with latitude? *American Naturalist* 178 (Suppl. 1), S80–96.
- Overgaard, J., Kristensen, T.N., Sørensen, J.G., 2012. Validity of thermal ramping assays used to assess thermal tolerance in arthropods. *PLoS ONE* 7, e32758.
- Pelini, S.L., Diamond, S.E., MacLean, H., Ellison, A.M., Gotelli, N.J., Sanders, N.J., Dunn, R.R., 2012. Common garden experiments reveal uncommon responses across temperatures, locations, and species of ants. *Ecology and Evolution* 2, 3009–3015.
- Piyaphongkul, J., Pritchard, J., Bale, J., 2012. Can tropical insects stand the heat? A case study with the brown planthopper *Nilaparvata lugens* (Stål). *PLoS ONE* 7, e29409.
- Porter, W.P., Gates, D.M., 1969. Thermodynamic equilibria of animals with environment. *Ecological Monographs* 39, 227–244.
- Powell, S.J., Bale, J.S., 2005. Low temperature acclimated populations of the grain aphid *Sitobion avenae* retain ability to rapidly cold harden with enhanced fitness. *Journal of Experimental Biology* 208, 2615–2620.
- R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. Version R 2.14.1. Available at <<http://www.R-project.org/>>.
- Ribeiro, P.L., Camacho, A., Navas, C.A., 2012. Considerations for assessing maximum critical temperatures in small ectothermic animals: insights from leaf-cutting ants. *PLoS ONE* 7, e32083.
- Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C., Pounds, J.A., 2003. Fingerprints of global warming on wild animals and plants. *Nature* 421, 57–60.
- Santos, M., Castañeda, L.E., Rezende, E.L., 2011. Making sense of heat tolerance estimates in ectotherms: lessons from *Drosophila*. *Functional Ecology* 25, 1169–1180.
- Sgro, C.M., Overgaard, J., Kristensen, T.N., Mitchell, K.A., Cockerell, F.E., Hoffmann, A.A., 2010. A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. *Journal of Evolutionary Biology* 23, 2484–2493.
- Shapley, H., 1920. Thermokinetics of *Liometopum apiculatum* Mayr. *Proceedings of the National Academy of Sciences of the United States of America* 6, 204–211.
- Shapley, H., 1924. Note on the thermokinetics of Dolichoderine ants. *Proceedings of the National Academy of Sciences of the United States of America* 10, 436–439.
- Sinervo, B., Méndez-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Resendiz, R., Martínez-Méndez, N., Calderón-Espinosa, M.L., Meza-Lázaro, R.N., Gadsden, H., Avila, L.J., Morando, M., De la Riva, I.J., Sepulveda, P.V., Rocha, C.F.D., Ibargüengoytia, N., Puntriano, C.A., Massot, M., Lepetz, V., Oksanen, T.A., Chapple, D.G., Bauer, A.M., Branch, W.R., Clobert, J., Sites, J.W., 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science* 328, 894–899.
- Sinervo, B., Miles, D.B., Martínez-Méndez, N., Lara-Resendiz, R., Méndez-De la Cruz, F.R., 2011. Response to comment on “Erosion of lizard diversity by climate change and altered thermal niches”. *Science* 332, 537.
- Sokal, R.R., Rohlf, F.J., 2012. *Biometry: The Principles and Practice of Statistics in Biological Research*. W. H Freeman, San Francisco.
- Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. *Science* 301, 65.
- Suggitt, A.J., Gillingham, P.K., Hill, J.K., Huntley, B., Kunin, W.E., Roy, D.B., Thomas, C.D., 2011. Habitat microclimates drive fine-scale variation in extreme temperatures. *Oikos* 120, 1–8.
- Sunday, J.M., Bates, A.E., Dulvy, N.K., 2011. Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences* 278, 1823–1830.
- SYSTAT, 2002. *TableCurve 2D 5.01 for Windows User's Manual*. SYSTAT Software Inc., California.
- Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., Chown, S.L., 2008. Thermal tolerance in a south-east African population of the tsetse fly *Glossina pallidipes* (Diptera, Glossinidae): implications for forecasting climate change impacts. *Journal of Insect Physiology* 54, 114–127.
- Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C., Chown, S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. *The Journal of Experimental Biology* 214, 3713–3725.
- Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T.J.C., Fromentin, J.-M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. *Nature* 416, 389–395.
- Warton, D.I., Duursma, R., Falster, D.S., Taskinen, S., 2011. (S)MATR: (Standardised) Major Axis Estimation and Testing Routines. R package version 3.2.4. Available at: <<http://CRAN.R-project.org/package=smatr>>.
- Weldon, C.W., Terblanche, J.S., Chown, S.L., 2011. Time-course for attainment and reversal of acclimation to constant temperature in two *Ceratitis* species. *Journal of Thermal Biology* 36, 479–485.
- Wilgenburg, E., Ryan, D., Morrison, P., Marriott, P.J., Elgar, M.A., 2006. Nest- and colony-mate recognition in polydomous colonies of meat ants (*Iridomyrmex purpureus*). *Naturwissenschaften* 93, 309–314.
- Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A., Langham, G., 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology* 6, e325.
- WMO, 2011. *Weather Extremes in a Changing Climate*. World Meteorological Organization, Switzerland. Available at: <http://www.wmo.int/pages/mediacentre/news/documents/1075_en.pdf>.