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ORIGINAL PAPER

Effects of desiccation and starvation on thermal tolerance and the heat-shock response in forest ants

Andrew D. Nguyen[1] · Kerri DeNovellis[1] · Skyler Resendez[2] · Jeremy D. Pustilnik[1] · Nicholas J. Gotelli[1] · Joel D. Parker[2] · Sara Helms Cahan[1]

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Abstract Temperature increases associated with global climate change are likely to be accompanied by additional environmental stressors such as desiccation and food limitation, which may alter how temperature impacts organismal performance. To investigate how interactions between stressors influence thermal tolerance in the common forest ant, *Aphaenogaster picea*, we compared the thermal resistance of workers to heat shock with and without pre-exposure to desiccation or starvation stress. Knockdown (KD) time at 40.5 °C of desiccated ants was reduced 6% compared to controls, although longer exposure to desiccation did not further reduce thermal tolerance. Starvation, in contrast, had an increasingly severe effect on thermal tolerance: at 21 days, average KD time of starved ants was reduced by 65% compared to controls. To test whether reduction in thermal tolerance results from impairment of the heat-shock response, we measured basal gene expression and transcriptional induction of two heat-shock proteins (*hsp70* and *hsp40*) in treated and control ants. We found no evidence that either stressor impaired the Hsp response: both desiccation and starvation slightly increased basal Hsp expression under severe stress conditions and did not affect the magnitude of induction under heat shock. These results suggest that the co-occurrence of multiple environmental stressors predicted by climate change models may make populations more vulnerable to future warming than is suggested by the results of single-factor heating experiments.

Keywords Ants · Heat-shock response · Desiccation · Heat-shock proteins · Starvation · Thermal tolerance

Abbreviations

KD	Knockdown
HSR	Heat-shock response
Hsp	Heat-shock protein gene
hsp70	Heat-shock protein 70 gene
hsp40	Heat-shock protein 40 gene
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase
Ef1β	Elongation factor 1 Beta
RT-qPCR	Real-time quantitative polymerase chain reaction
GLM	Generalized linear model
ANOVA	Analysis of variance
LT_{50}	Median lethal time

Introduction

Temperature increases are projected to alter species distributions and abundances, particularly for ectotherms (Diffenbaugh and Field 2013). Whether a population is resilient in the face of higher temperatures is likely to depend on the temperature differential between the local thermal environment and the organism's critical thermal maximum, known as the thermal safety margin (Deutsch et al. 2008). Species with smaller thermal safety margins are predicted to be at risk of population declines as they approach or exceed their upper thermal limits (Deutsch et al. 2008; Diamond et al. 2012; Clusella-Trullas et al. 2011; Kellermann et al. 2012). In contrast, species with larger thermal safety margins may

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✉ Andrew D. Nguyen
anbe642@gmail.com

[1] Department of Biology, University of Vermont, 109 Carrigan Drive, Burlington, VT 05405, USA

[2] Biological Sciences, State University of New York Plattsburgh, 101 Broad St, Plattsburgh, NY 12901, USA

benefit from additional warming, because they typically operate at sub-optimal temperatures and would thus be shifted up their performance curve toward optimal operating body temperature (Deutsch et al. 2008; Diamond et al. 2012; Clusella-Trullas et al. 2011; Kellermann et al. 2012). However, taxa with larger thermal safety margins generally occupy locations with high-temperature variation and extreme high temperatures may cause overheating (Kingsolver et al. 2013). In addition, species that overwinter may be at risk of mortality during the winter months, because warming can impact the microclimate and expose quiescent organisms to higher temperatures (Williams et al. 2015).

Critical thermal limits may also vary with environmental context, enhancing or reducing the thermal safety margin (Cahill et al. 2013; Chahal and Dev 2013; Duffy et al. 2015). Although thermal tolerances are typically measured in animals maintained under ideal conditions, extreme heat is projected to co-occur with reduced precipitation (Mueller and Seneviratne 2012), which may result in species simultaneously encountering both thermal and desiccation stresses. Furthermore, temperature may act indirectly through shifts in prey availability or interspecific competition, potentially leading to nutritional stress (Araújo and Luoto 2007).

The combined effect of multiple environmental stressors ultimately depends on the underlying molecular pathways used to combat their effects (Sinclair et al. 2013). If different stressors activate the same response pathways, exposure to one stressor can enhance resistance to another in a cross-protective manner (cross tolerance; Todgham and Stillman 2013; MacMillan et al. 2009). In Antarctic midges, for example, desiccation provided cross protection against heat stress (Benoit et al. 2009). One molecular pathway likely to show a generalized response is the heat-shock response (HSR), which senses and repairs protein damage (Richter et al. 2010). However, if different stressors activate distinct molecular pathways, exposure to one may have no effect on response to the other, or may even decrease tolerance (cross-susceptibility) due to the energetic demands of responding to multiple stressors simultaneously (Sinclair et al. 2013; Todgham and Stillman 2013). In fruit flies, desiccation stress reduces upper thermal limits across a broad range of sub-lethal temperatures (Da Lage et al. 1989). Similarly, starvation has been found to either have no effect (Bubliy et al. 2012b) or a cross-susceptibility effect on thermal tolerance (Floyd 1985).

Ants are a good system to explore the impact of different stressors on thermal limits, because they have colonized and inhabit diverse environments (Moreau and Bell 2013; Economo et al. 2015). Many species have a broad geographical range and are exposed to considerable environmental variation (Sanders et al. 2007; Dunn et al. 2009; Kaspari et al. 2015). Foraging activity is sensitive to temperature (Albrecht and Gotelli 2001; Wittman et al. 2010), soil moisture (Gordon 2013), available resources (Stuble et al. 2013), and species interactions (Rodriguez-Cabal et al. 2012) that altogether impact food intake for the whole colony. Ants are experimentally tractable for studies of physiological studies in response to multiple environmental conditions. Although ants likely face multiple stressors, we have very little understanding of how these additional sources of environmental stress such as desiccation and starvation are likely to impact thermal tolerance.

In this study, we tested how desiccation and nutritional stressors affect thermal tolerance in a common forest ant, *Aphaenogaster picea*. In a static heat-shock experiment, we compared knock-down (KD) times of workers maintained in control conditions to those exposed to either desiccation or starvation stress at progressive levels of severity. To determine whether changes in thermal tolerances were due to repression or enhancement of the heat-shock response (HSR), we quantified baseline and transcriptional activation of two representative genes: *hsp70* and *hsp40*. We found that desiccation and starvation did not alter the HSR, but both diminished thermal limits across all levels of severity.

Materials and methods

Natural history of *Aphaenogaster picea*

Aphaenogaster picea is a ground-dwelling species that occurs in mesic deciduous forests in the eastern United States from the high elevations of Virginia to Maine (DeMarco and Cognato 2015). Across their distribution, mean annual temperature ranges from 5 to 14 °C, but leaf litter temperatures in the summer can be as high as 40 °C, while below-ground temperatures may remain at 20 °C (Lubertazzi 2012). Colonies are comprised of roughly 180–1000 individuals that nest within the soil and coarse woody debris (Lubertazzi 2012). Foragers collect and disperse seeds containing elaiosomes (Warren et al. 2011), which provide the colony with a nutritional benefit (Morales and Heithaus 1998; Clark and King 2012).

Over the last 40 years, elevational limits have shifted upwards at the warm edge of their geographical range, suggesting that contemporary environmental change may already be affecting local populations (Warren and Chick 2013). Seed collection and dispersal by *A. picea* are sensitive to soil surface temperatures (Warren et al. 2011; Stuble et al. 2014) and soil moisture (Warren et al. 2010). Increasing temperatures have also led a phenological mismatch with ant-dispersed seed plants, as well as increased competitive pressure from more thermophilic native and

invasive ant species (Bewick et al. 2014; Warren and Bradford 2014).

Field collections and rearing ant colonies

Whole queenright *A. picea* colonies consisting of eggs, larvae, pupae, and adult ants were collected from coarse woody debris and leaf litter between June and July 2013 at East Woods (N 44.440 W 73.197) located near the University of Vermont, in South Burlington, Vermont. Colonies were maintained in the laboratory at 25 °C constant temperature in 22 × 16 cm plastic containers lined on the sides with Insect-a-slip (Bioquip) to prevent escape, and filled to a depth of 50 mm with sand. We supplied each colony with two test tubes (160 × 140 mm) half filled with water that were plugged with a cotton wad. The saturated cotton provided water and ants nested in the opening of the tube. Colonies were fed approximately 100 µL of 20% honey water and one bisected mealworm three times a week.

Stress pre-treatment: desiccation

To determine the effect of desiccation on thermal tolerance, we placed sets of ten adult nestmate workers either into a 15 mL conical tube filled at the 7 mL mark with desiccant (10% relative humidity) separated by cotton to avoid contact, or a 15 mL conical tube half-filled with water capped with a cotton plug. To characterize the time-course of survival under continuous desiccation stress, we recorded survival status every half hour over an 8-h period for four colony-level replicates. Based on the survival analysis, we chose three desiccation timepoints to represent mild, moderate, and severe sub-lethal desiccation stress and repeated the treatments for a new set of colonies (Fig. 1a). For each desiccation and control treatment and across each timepoint, we treated and then sampled ten ants per colony for thermal tolerance assays (see below) with seven colony-level replicates; for five of these colonies, an additional 4 ants for each treatment group were sampled for gene expression. To estimate the extent of desiccation, we measured for each set of four ants, the pooled initial wet weight (W_i), pooled final wet weight (W_f), and pooled final dry weight (D_f) to calculate the % initial water content as follows:

$$\%\ \text{Initial Water Content} = 100 - \left(\frac{W_i - W_f}{W_i - D_f}\right) \times 100.$$

Stress pre-treatment: starvation

To determine the effect of starvation on thermal tolerance, we established two dietary treatments (starved and fed) for five timepoints spread over 3 weeks (days 1, 3, 7, 14, and 21). Forty ants were randomly assigned to each dietary treatment and timepoint. Starved ants were reared in a cotton-plugged water tube with no access to food. Dead ants were removed daily to prevent cannibalism. Control ants were reared in an identical rearing tube but with access to 20% honey water and meal worms every 2 days. We tracked survival at each timepoint. At each timepoint, we used ten ants from each treatment to assay for thermal tolerance with a total of ten colony-level replicates and four ants per treatment group for gene expression analyses for five of the colonies. To quantify condition, we measured the pooled dry mass (to the nearest 0.01 mg) of ants after thermal tolerance experiments (see below). To control for ant size, we regressed the mean head widths (mm) against mean dry mass and used the standardized residual

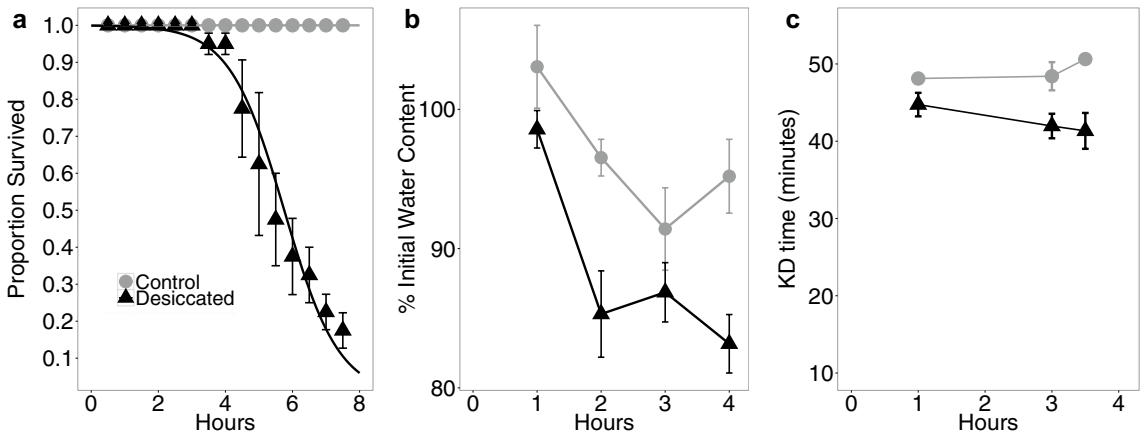

Fig. 1 Effect of desiccation (*black triangles*) on **a** survival, **b** initial water content, and **c** thermal tolerance relative to control ants (*gray circles; n* = 7 colony replicates per timepoint and treatment). Survival under desiccation stress was determined over an 8 h period and water content was measured from 1 to 4 h; we selected hours 1, 3, and 3.5 for subsequent static heat-shock treatment (40.5 °C) and *error bars* represent ± 1 standard error of the mean

(mean = 0 and variance = 1) as a measure of size-corrected dry mass. Head width was measured as the maximum distance in mm (to the nearest 0.01) between the eyes using the ImageJ software.

Measuring thermal tolerance

We used a static heat-shock protocol (Terblanche et al. 2011) to avoid the confounding issue of ongoing desiccation associated with a slow ramping protocol (Rezende et al. 2011). Preliminary trials revealed that 40.5 °C yielded KD times under an hour and that ants are able to recover from and survive for at least a few days. For each set of ten nest-mate workers associated with each timepoint and treatment (see pre-treatments above), pairs of randomly selected workers were placed in five separate 5mL glass screw-cap vials. Three of the five vials were heat shocked by fully submerging the vial at 40.5 °C in a pre-set Thermo Neslab EX17 heating water bath, while the remaining two vials were simultaneously held at room temperature (25 °C). Heat-shocked ants were observed continuously at a temporal resolution of roughly 10 s until KD, defined as loss of activity (Terblanche et al. 2011). To avoid bias, we measured KD times without prior knowledge of the treatment groups.

Measuring the HSR

For the subset of colonies that we sampled to measure the HSR, the ants were exposed to identical heat-shock and control conditions as those in the thermal tolerance assay, but were removed at 25 min and flash-frozen in liquid nitrogen and stored at −80 °C. Ants were sampled regardless of KD status, and preliminary analyses showed that ants were able to induce *hsp70* and *hsp40* at the 25 min mark.

For each gene expression sample, two of the four flash-frozen ants were pooled and homogenized in a bullet blender homogenizer (Next Advance Inc., USA) at top speed (10) with 1.4 mm zirconium silicate beads (Quackenbush Co., Inc, USA). RNA was isolated with RNAzol (Molecular Research, USA) and then purified with the RNeazy Micro Kit (Qiagen, USA), both following the manufacturers' instructions. 100 ng of RNA was converted to cDNA with the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, USA) following the manufacturer's instructions.

The gene expression patterns of *hsp70* (*hsc70-4* h2 orthologue) and *hsp40* were quantified using previously developed primers (Nguyen et al. 2016) with RT-qPCR on a StepOnePlus instrument (Applied Biosystems, USA). Each sample was run in triplicate in 20 µL reactions comprised of 2 ng of template, 250 nM of forward primer and of 250 nM reverse primer, and 1× Power SYBR® PCR master mix (Life Technologies, USA). Reactions were incubated at 95 °C for 2 min and then underwent 40 cycles of 95 °C for 15 s followed by 60 °C for 60 s. Amplicon specificity was assessed with a melt-curve analysis. We used the geometric mean of *ef1β* and *gapdh*as house-keeping genes, which had the lowest measure of variation according to NormFinder (stability = 0.23; Andersen et al. 2004). We used $2^{-\Delta\Delta CT}$ as the measure of basal gene expression and fold induction under heat shock (Livak and Schmittgen 2001). For basal gene expression, $2^{-\Delta\Delta CT}$ was calculated relative to time and colony-matched controls (water-plugged treatment or fed treatment). Fold induction of heat-shocked ants was calculated relative to time and colony-matched controls (room temperature, 25 °C).

Statistical analyses

All statistical analyses were performed in R (version 3.2; R Core Team 2016). In all of our statistical analyses, colony was treated as an independent block for estimating treatment effects; including colony as a random effect achieved similar results and we present only the findings from fixed effects models. Survival was analyzed with a GLM, which fits a logistic relationship between the proportion of individuals surviving and time (hours or days). Lethal time at 50% (LT_{50}) was estimated from GLM-fitted models with the *dose.p()* function in the MASS package. We determined the effect of time, treatment, and time × treatment interaction on KD time or Hsp gene fold induction with an ANCOVA. To avoid over-fitting statistical models, we used a backwards AIC selection criterion with the *stepAIC()* function in the MASS package (Venables and Ripley 2002). In models of *Hsp* expression (basal and induction), values were log10-transformed to meet the assumptions of normality. To determine significant differences in Hsp basal gene expression between pre-treatments (desiccation or starvation) with controls for each timepoint, we used a one-sample *t* test to test for significant differences from zero.

Results

Effect of desiccation on thermal tolerance and HSR

Ants in the desiccation treatment experienced 100% mortality after 8 h ($LT_{50} = 5.7 \pm 0.1$ h; GLM: Desiccation $z = -1.23$), during which time there was no mortality of control ants (Fig. 1a). The onset of mortality for desiccated ants was at ~4 h (Fig. 1a). Although water content of ants in both treatments decreased through time, (ANOVA, $F_{1,77} = 20.73$, $p < 0.001$), desiccated ants had lower water content than controls ($F_{1,77} = 20.09$, $p < 0.001$; Fig. 1b),

with most water loss occurring in the first hour. With heat shock, the KD time of desiccated ants was reduced within the first hour by 6% compared to controls ($F_{1,58}$ = 25.21, p < 0.001; Table 1) and did not decrease further with through time (Fig. 1c).

Pooled among times, desiccated and control treated ants did not differ in overall basal expression of *hsp70* (one-sample *t* test, t = 1.03, df = 12, p = 0.32) or *hsp40* (t = 0.41, df = 12, p = 0.69). When timepoints were analyzed separately, desiccated ants also did not differ significantly from controls in *hsp70* basal gene expression (Fig. 2a), but desiccated ants had 1.3-fold higher *hsp40* basal expression at the 3.5 h (one-sample *t* test, t = 4.54, df = 4, p < 0.05, Fig. 2b). In response to heat shock, desiccated and control ants induced *hsp70* and *hsp40* to similar levels (Fig. 2b, d; Table 1).

Effect of starvation on thermal tolerance and HSR

Starved ants died (LT_{50} = 24.3 ± 1.1 days) sooner than control ants (LT_{50} = 35.5 ± 3.9 days; GLM: Dietary treatment z = −3.138, p < 0.001; Fig. 3a). Residual dry mass decreased more rapidly in starved than control treated ants (Treatment × time interaction, $F_{1,59}$ = 4.18, p < 0.05, Fig. 3b). By day 21, starved ants declined in residual dry mass to 83.5% of controls (Fig. 3b). KD time decreased significantly with progressive starvation (Treatment × time interaction, $F_{1,45}$ = 38.68, p < 0.001, Fig. 3c; Table 1). By day 21, KD time of starved ants declined to 35% of the controls (Fig. 3c).

Starved and control treated ants did not differ overall in basal expression of *hsp70* (one-sample *t* test, t= -0.34, df = 23, p = 0.73) and *hsp40* (t = 0.92, df = 23, p = 0.36). When analyzed separately by day, however, starved ants significantly down-regulated *hsp70* 1.66-fold on day 3 (t = −4.08, df = 4, p < 0.05), but up-regulated it 1.4-fold on day 21 (t = 4.27, df = 4, p < 0.05; Fig. 4a). Starved ants up-regulated h*sp40* twofold more than controls on day 1 (t = 4.24, df = 4, p < 0.05; Fig. 4c). Starved and control ants induced *hsp70* and *hsp40* ($F_{1,28}$ = 0.00, p = 0.98; Fig. 4b, d) to similar levels, but the induction of *hsp70* declined with time in both groups (ANCOVA, $F_{4,28}$ = 8.01, p < 0.001; Fig. 4b).

Discussion

Environmental stressors often act in concert, either increasing tolerance through cross protection or decreasing tolerance through cross susceptibility (Todgham and Stillman 2013). In this study, we found support for cross-susceptibility of thermal limits to desiccation and starvation (Figs. 1c, 3c). The magnitude of reduced thermal tolerance reflected additive and interactive effects of desiccation and starvation on survival (Figs. 1a, 3a) and physiological condition (Figs. 1b, 3b). The effect of desiccation stress was relatively mild, but starvation produced a marked effect over time, decreasing KD time by over 50% under severe stress conditions. If such stressors are experienced in the field, the thermal safety margin of this species under projected climate change scenarios for the northeastern US may be significantly smaller than that predicted by temperature alone (Clusella-Trullas et al. 2011).

The cross effects of desiccation stress on thermal tolerance may depend on the timing and magnitude of stressors applied (Kingsolver and Woods 2016; Gunderson et al. 2016). Simultaneous application of drying and warming often diminish thermal tolerances (Maynard Smith 1957; Da Lage et al. 1989; Holmstrup et al. 2002; Bubliy et al. 2012a), and we also found cross-susceptibility between desiccation and rapidly-applied heat under sequential application of stressors. However, under slow heating protocols, desiccation has not been found to depress thermal tolerance

Table 1 Summary of statistical analyses for survival, thermal tolerance(KD time), condition (water content, dry mass), and heat-shock response (*hsp70* and *hsp40* expression)

Experiment	Sources of variation	Survival	KD-time	Water content	Dry mass	Hsp70		Hsp40	
						Basal	Induction	Basal	Induction
Desiccation	Colony	++							ns
	Treatment (desiccated/control)	+++	+++	+++				+	ns
	Time (h)	+++		+++				+	ns
	Treatment × time						ns		ns
Starvation	Colony	+	+++		+++	+	ns	+	ns
	Treatment (starved/control)	++	+++		ns	+	+++	+	
	Time (days)	+++	+++		+		ns		
	Treatment × time		+++						

'+': p < 0.05; "++": p < 0.01; "+++": p < 0.001; *ns* not significant. Empty cells represent predictors that were not retained from AIC model selection

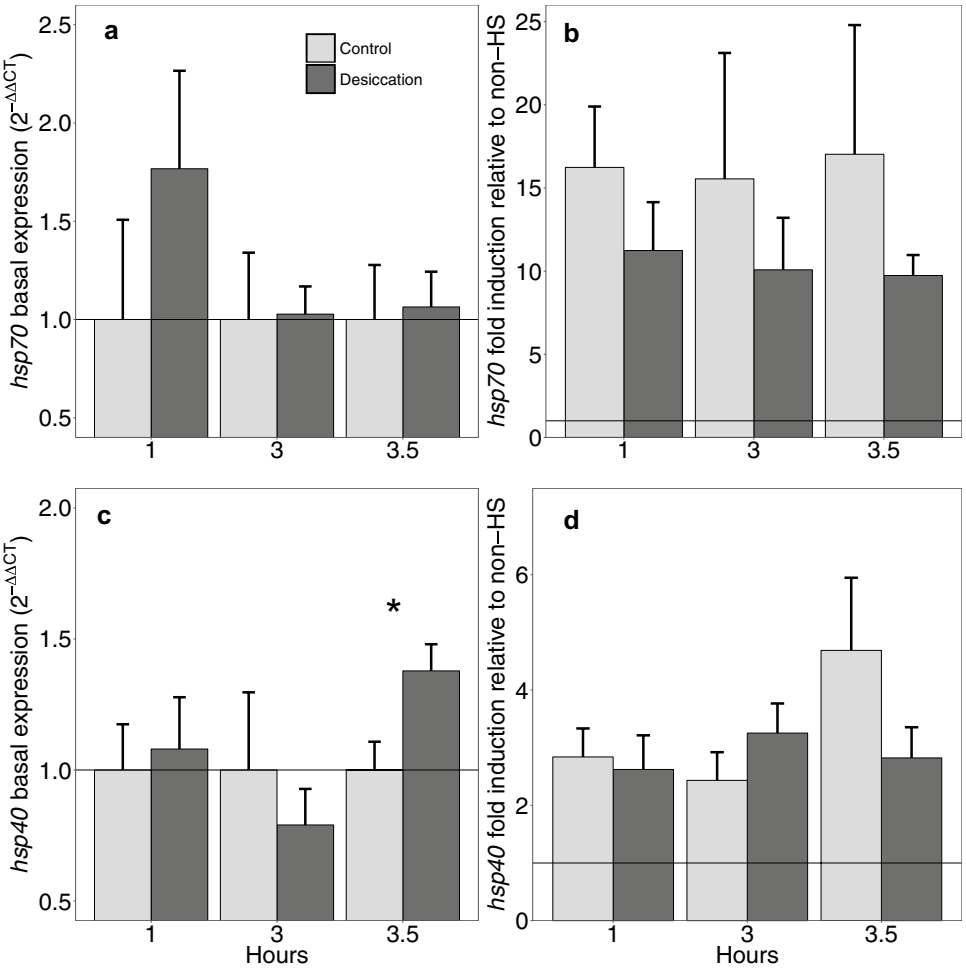

Fig. 2 Effect of desiccation (*dark gray*) on *hsp70* and *hsp40* gene expression. **a**, **c** Show basal gene expression between control and desiccation treatments. **b**, **d** Show the extent of fold induction of HS relative to non-HS ants between control and desiccation treatments. For each treatment and timepoint, there were 4–5 colony-level replicates and *error bars* represent ± 1 standard error of the mean. For basal gene expression, $2^{-\Delta\Delta CT}$ was calculated relative to time and colony-matched controls (hydrated treatment) and the controls themselves are represented by scaling the mean to 1 with the standard error of the mean expressed as among colonies. For fold induction of heat-shocked ants, $2^{-\Delta\Delta CT}$ was calculated relative to time and colony-matched controls (room temperature, 25 °C)

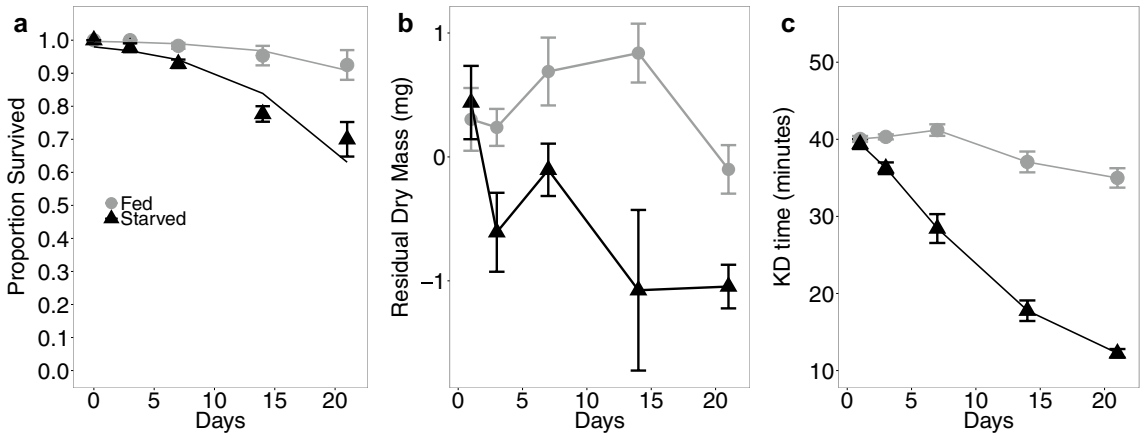

Fig. 3 Effect of starvation (*black* triangles) on **a** survival, **b** residual dry mass, and **c** thermal tolerance relative to control ants (*gray circles*). Survival under starvation stress was determined over 21 days and we selected 1, 3, 7, 14, and 21 day timepoints for subsequent static heat-shock treatment (40.5 °C) and measured residual dry mass. Residual dry mass represents the size-corrected dry mass, which was obtained by extracting the residuals from a linear regression between dry mass and head width. Residual dry mass was standardized, such that the mean = 0 and variance = 1. For each treatment and timepoint, there were ten colony-level replicates and *error bars* represent ± 1 standard error of the mean

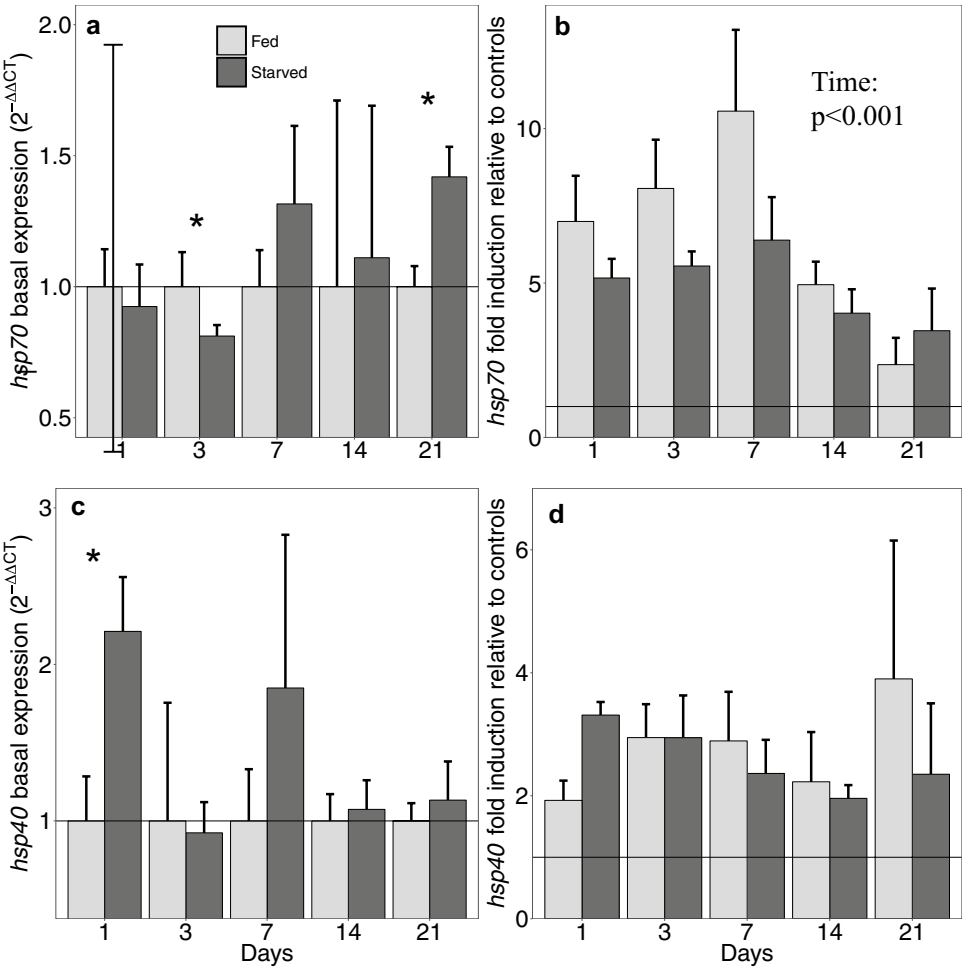

Fig. 4 Effect of starvation (*dark gray*) on *hsp70* and *hsp40* gene expression. **a**, **c** Show basal gene expression between control and starvation treatments. **b**, **d** Show the extent of fold induction of HS relative to non-HS ants between control and starvation treatments. For each treatment and timepoint, there were 4–5 colony-level replicates and *error bars* represent ± 1 standard error of the mean. For basal gene expression, $2^{-\Delta\Delta CT}$ was calculated relative to time and colony-matched controls (fed treatment) and the controls themselves are represented by scaling the mean to 1 with the standard error of the mean expressed as among colonies. For fold induction of heat-shocked ants, $2^{-\Delta\Delta CT}$ was calculated relative to time and colony-matched controls (room temperature, 25 °C)

(Terblanche et al. 2011). In fact, with sufficient recovery time between these two stressors (Bubliy et al. 2012b) or slow application of desiccation (Benoit et al. 2010), desiccation conferred cross tolerance against heat stress.

Dehydration can either enhance or inhibit thermal defense mechanisms (Benoit et al. 2009; Bubliy et al. 2012b). Protein denaturation resulting from any type of perturbation, including desiccation stress, elicits the HSR (Kültz 2003, 2005) by rapidly up-regulating Hsps (Craig and Gross 1991; Hayward et al. 2004; Morris et al. 2013; Mizrahi et al. 2010). Hsps that remain induced after the stressor subsides offer a short-term "hardening" effect that can increase survival in the face of subsequent exposure to the same stressor (Cavicchi et al. 1995), and can also potentially cross protect against other stressors (Bubliy et al. 2012b). Consistent with hardening, we found that desiccation increased basal expression of *hsp40* at the highest exposure (Fig. 2c). Despite this effect, however, increased *hsp40* expression did not result in enhanced upper thermal limits.

In comparison with desiccation, starvation imposed greater cross-susceptibility to thermal damage. Consistent with previous work (Bubliy et al. 2012b; Overgaard et al. 2012), thermal tolerance was not affected by mild starvation, but with increasing exposure time, thermal tolerance declined 65% compared to fed workers of the same age (Fig. 3b). Because thermal tolerance is typically estimated for lab-acclimated organisms provided with *ad libitum* food, our result suggests that such experiments may substantially overestimate thermal tolerance expressed by individuals in the field (Tagliarolo and McQuaid 2016). Such an effect may be particularly ecologically relevant in ants, because foraging outside the nest is performed primarily by the oldest and most resource-depleted individuals (Howard and Tschinkel 1980; Tschinkel 1998; Tripet and Nonacs 2004; Dussutour et al. 2016).

Thermal defenses are metabolically expensive (Bettencourt et al. 2008; Hoekstra and Montooth 2013), leading to the expectation that HSR activation would lessen as internal energy reserves become depleted. In both dietary groups, both *hsp70* gene fold induction and KD time declined over the course of the experiment, potentially reflecting decreased ability to mount a sufficient response as individuals aged (Bowler and Terblanche 2008). However,

starvation was associated with transient increases in basal Hsp gene expression, and starved and fed ants invested similarly in Hsp gene up-regulation in response to heat stress (Fig. 4), suggesting that allocation of energy to protein protection is not impacted under low-resource conditions. As with desiccation, starvation-induced increases in basal Hsp gene expression at early and late timepoints were not associated with increases in KD time (Fig. 4a, c). It is possible that other molecular pathways that contribute to coping with stress that were not measured here, such as damage repair, redox regulation, and energy metabolism, are depressed by starvation and outweigh the slight increase in the Hsp response (Zinke et al. 2002; Kültz 2005).

Taken together, the results of this study suggest that single-stressor assays may not be a reliable method for estimating thermal tolerance, and thus the capacity to withstand additional warming. Future climate change is likely to impose simultaneous combinations of environmental stressors such as temperature, desiccation, and starvation. Each of these is likely to impose stress on individual and colony-level performance and elicit physiological defenses; however, in addition to their independent effects, their interaction has the potential further reduce temperature tolerances. To improve species forecasts, models of physiological responses to climate change should account for these diverse sources of stress (Terblanche et al. 2007).

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Compliance with ethical standards

Conflict of interest No competing interest declared.

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