

Heat hardening in a tropical lizard: geographic variation explained by the predictability and variance in environmental temperatures

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Summary

1. Over the coming decades, our planet will experience a dramatic increase in average temperatures and an increase in the variance around those temperatures leading to more frequent and harsher heat waves. These changes will impact most species and impose strong selection on physiological traits.

2. Rapid acclimation is the most direct way for organisms to respond to such extreme events, but we currently have little understanding of how the capacity to mount such plastic responses evolves. Accordingly, there is some urgency to determine how the physiological response to high temperatures varies within species, and how this variation is driven by the environment.

3. Here, we investigate heat-hardening capacity – a rapid physiological response that confers a survival advantage under extreme thermal stress – across 13 populations of a rain forest lizard, *Lampropholis coggeri*, from the tropics of north-eastern Australia.

4. Our results reveal that heat hardening is constrained in these lizards by a hard upper thermal limit for locomotor function (approximately 43 °C). Further, hardening response shows strong geographic variation associated with thermal environment: lizards from more predictable and more seasonal thermal environments exhibited greater hardening compared with those from more stochastic and less seasonal habitats. This finding – that predictability in thermal variation influences hardening capacity – aligns closely with theoretical expectations.

5. Our results suggest that tropical species may harbour adaptive variation in physiological plasticity that they can draw from in response to climate change, and this variation is spatially structured in locally adapted populations. Our results also suggest that, by using climatic data, we can predict which populations contain particular adaptive variants; information critical to assisted gene flow strategies.

Key-words: heat shock, local adaptation, plasticity, thermal limits

Introduction

As anthropogenic climate change advances, there is increasing concern about the impacts of this sudden and accelerating climatic shift on the Earth's biodiversity (e.g. Thomas *et al.* 2004; Bellard *et al.* 2012). The majority of these concerns centre on the rapid increase in temperature and temperature variance that is already underway (Meehl *et al.* 2007). Understanding how organisms will respond to climate warming requires not only

appreciating the breadth of adaptive variation within species, but also how it is spatially structured throughout their distribution (Hoffmann & Sgrò 2011). Many species, particularly those with broad geographic ranges, have populations living under a diverse array of climatic conditions. The selective pressure imposed by climate, and the capacity of populations to adapt to these varying conditions may be revealed by examining both the geographic variation in an organism's physiological traits, as well as in the flexibility of these traits to shift with local conditions (e.g. Hoffmann *et al.* 2003; Latimer, Wilson & Chenoweth 2011).

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There has been substantial work on geographic variation in critical thermal limits (see reviews in Chown 2001; Angilletta 2009). The focus so far has largely been on variation across, rather than within, species and, when within species, across large geographic distances rather than at the scales relevant to gene flow (although see Muñoz *et al.* 2014; Rezende, Castañeda & Santos 2014). Recent meta-analyses of this body of work have found low levels of variation in heat tolerance across broad geographic ranges, suggesting a limited capacity for upper thermal limits to shift under climate warming (Sunday, Bates & Dulvy 2011; Araújo *et al.* 2013).

We have substantially less information about intraspecific variation in the plasticity of individuals' thermal limits. As with the work on thermal limits, the majority of work on heat tolerance plasticity has focused on cross-species comparisons (e.g. Bilyk & Devries 2011; Nyamukondiwa *et al.* 2011; Overgaard *et al.* 2011), rather than variability within species (although see Hoffmann, Sørensen & Loeschcke 2003; Sgrò *et al.* 2010 and references therein for the exceptions to this trend, primarily involving *Drosophila*). Thus, there is presently a gap in our understanding of how adaptive variation in the plasticity of physiological traits is distributed across climatically diverse landscapes.

It is, nonetheless, clear that physiological plasticity is a common phenomenon. Plastic shifts in physiological traits can manifest over the course of hours to a few days ('hardening' or 'shock response'; Bowler 2005), or more gradually over a few weeks to months ('acclimation'), and both shifts have been observed in a wide variety of ectotherms (Seebacher 2005). Whereas gradual acclimation responses confer resilience to slower shifts in thermal environment (such as seasonal changes), hardening responses likely increase fitness under short, extreme climatic events (such as heat waves; Angilletta 2009). Loeschcke & Hoffmann (2007), for example, showed that heat-hardened *Drosophila* were more active under heat-wave conditions than were control conspecifics, suggesting that heat hardening can provide a fitness advantage during periods of unusually high temperature. Given that one of the acute impacts of climate change will be an increasing frequency and severity of unpredictable extreme temperature events (Parmesan, Root & Willig 2000), heat hardening seems a particularly pertinent trait to investigate.

Heat hardening is not, however, a universally beneficial response: its adaptive value is thought to be highly contingent on local conditions. Mounting a hardening response can be costly, particularly in the production and maintenance of proteins associated with heat shock (Somero 2002). Hence, to avoid these costs and to avoid inappropriate responses, heat hardening should be dampened in thermally unpredictable environments (Huey & Kingsolver 1989; Gilchrist 1995; Somero 2002). When heat-shock responses are mounted, their magnitude should further depend on an individual's starting point. There is a clear expectation, both on biochemical grounds as well as from broad comparative studies (Chown 2001; Hochachka &

Somero 2002; Stillman 2003; Hoffmann, Chown & Clusella-Trullas 2012) that a species' upper critical thermal limit faces strong constraints. Thus, an individual starting close to this hypothetical limit may only be able to mount a small heat-hardening response relative to an individual starting distant from this hypothetical limit (e.g. Stillman 2003).

Work on geographic variation in heat hardening to date has predominantly focused on invertebrates (particularly *Drosophila*, for reviews see Hoffmann, Sørensen & Loeschcke 2003; Sgrò *et al.* 2010). In this study, we examine patterns of heat hardening in the tropical scincid lizard, *Lampropholis coggeri*, from the Wet Tropics rain forest of north-eastern Australia. We demonstrate that there is substantial geographic variation in the magnitude of heat hardening across 13 populations, but that all populations are confronted with a common upper tolerance limit. Finally, we show that almost all of the across-population variation in hardening capacity is explained by the predictability and seasonality of maximum temperature in the lizards' environment.

Materials and methods

STUDY SYSTEM AND SPECIES

The sunskink *Lampropholis coggeri* is a small (maximum snout-vent length of 45 mm), diurnal lizard restricted to the Wet Tropics rain forests of north-east Queensland, Australia (Australian Reptile Online Database 2014; Fig. 1). These skinks thermoregulate by alternately basking in direct sun and foraging in and under leaf litter. They are especially common in edge habitats, namely in canopy gaps and along road and forest edges. The species has a low dispersal rate: approximately $80 \text{ m} \cdot \text{gen}^{-0.5}$ (Singhal & Moritz 2012). Across the Wet Tropics *L. coggeri* has two deeply divergent lineages: a northern and southern lineage which likely represent different species (Bell *et al.* 2010). To ensure intraspecific sampling, we focused our study on the southern lineage only, sampling from Hervey Range in the south, north to the Kirrama Range (Fig. 1). Within this southern lineage of *L. coggeri*, we sampled lizards from 13 populations (Table 1). Pairwise geographic distances between these populations range from 1.8 to 115 km (Table S1, Supporting information).

MEASUREMENT OF HEAT HARDENING

Following capture by hand, skinks were housed individually in long plastic tubs (340 × 120 × 160 mm) at the James Cook University animal research facility. Each tub was coated with shredded paper (mimicking leaf litter), and an 84-mL container coated with moist vermiculite to provide the skinks with two types of shelter and water. Tubers were maintained on heat racks that produced a thermal gradient (21–40 °C) inside each tub, allowing the skinks to thermoregulate. Lighting followed the natural 10 : 14 light to dark cycle and was supplemented with ultraviolet light for 2 h each morning. Skinks were fed crickets every second day, but no animals were fed in the morning prior to testing. All skinks were tested within a fortnight of being collected from the field.

High temperature hardening was measured as the change in an individual's upper thermal limit following an exposure to high temperature. In practice, this meant that we measured the upper

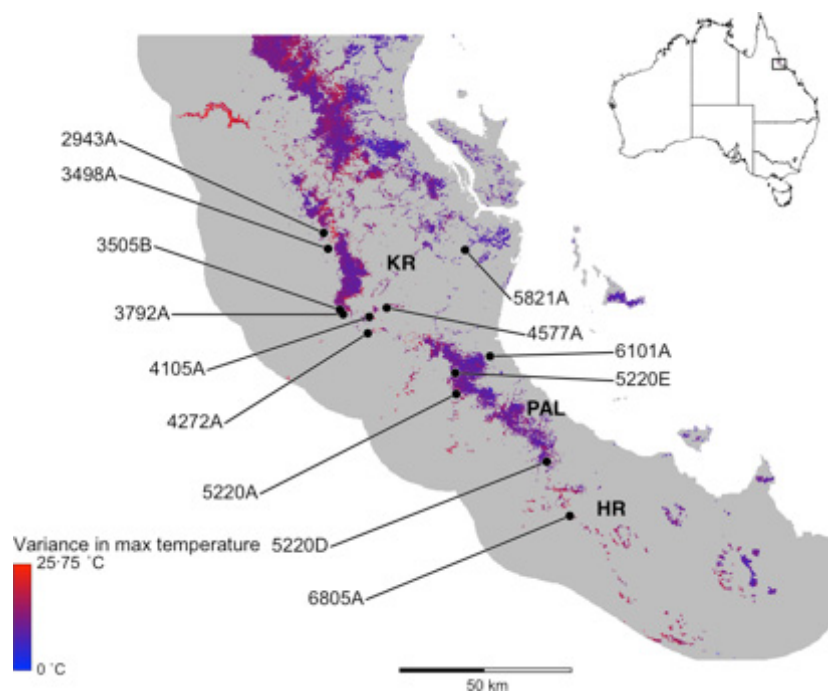


Fig. 1. Map of our study area showing the 13 sampled populations. Coloured regions represent rain forest habitat, and the colour scale describes the variation in daily maximum temperatures (from 1990 to 2009) at each pixel. Sample code 5220A is the Paluma population, and 6805A is the Hervey Range population. The major mountain ranges are also labelled: Kirrama Range (KR); Paluma Range (PAL); and Hervey Range (HR).

Table 1. Populations sampled, location, sample sizes and mean mass and ΔCT (i.e. heat-hardening response) of lizards at each site

Site ID	Latitude	Longitude	<i>n</i>	Mean mass, g (SD)	mean ΔCT °C (SD)
6805A	-19.3629	146.4769	14	1.27 (0.27)	1 (0.67)
5220D	-19.2093	146.4113	13	0.32 (0.05)	0.18 (0.33)
5220A	-19.0178	146.1549	9	1.21 (0.06)	0.31 (0.52)
5220E	-18.9583	146.1523	16	0.31 (0.07)	0.29 (0.26)
6101A	-18.9106	146.2508	11	0.79 (0.42)	0.45 (0.31)
4272A	-18.8458	145.9041	10	1.05 (0.23)	0.04 (0.48)
4105A	-18.7992	145.9086	16	0.4 (0.2)	0.22 (0.52)
3792A	-18.7921	145.8345	5	0.96 (0.33)	-0.16 (0.23)
3505B	-18.7788	145.8250	9	0.96 (0.28)	0.54 (0.42)
4577A	-18.7736	145.9579	10	1.15 (0.19)	0.17 (0.34)
5821A	-18.6096	146.1800	8	0.56 (0.41)	0.3 (0.23)
3498A	-18.6062	145.7915	14	0.89 (0.6)	0.89 (0.71)
2943A	-18.5614	145.7789	10	0.48 (0.41)	0.78 (0.42)

critical thermal limit (CT_{max}) for each individual twice (separated by a set period of time; see 'Timing of hardening response' below), and calculated the difference between these two measures (ΔCT) as our measure of hardening. Measurement of CT_{max} was achieved as follows: First, we placed skinks individually in 50 mL plastic conical tubes with the tubes' lids fastened. The skinks were then incubated for 20 min at 35 °C to ensure that all animals started the trial at the same body temperature. Tubes were removed from the incubator one at a time to begin each skink's trial. The tube's lid was replaced with a lid that had a thermocouple probe passing through its centre, allowing us to measure the temperature inside the tube. We measured air temperature inside

the tube rather than the skink's cloacal temperature because the insertion of a thermocouple probe into the cloaca of such small organisms (mean 1.2 g, max 2.7 g) severely impedes their righting ability. Thus, our temperature measure may not precisely reflect the internal temperature of the lizard, but should be closely correlated with it. The tube was submerged in an insulated box containing water at 35 °C, and we then increased the temperature in the tube at a rate of one degree per minute (monitored by continuous-time, digital temperature readings) by adding hot water to the insulated box. Whilst increasing the temperature, we frequently flipped the skink onto its dorsal surface (approximately every 10 s) by rotating the tube. The temperature at which the skink could no longer right itself was recorded as its CT_{max} . When a skink reached its CT_{max} , we immediately dipped the skink in a 20 °C water bath to rapidly cool the animal. All skinks recovered from the CT_{max} trials.

TIMING OF HEAT-HARDENING RESPONSE

Because heat-hardening trials have not been conducted in this species, we first sought to confirm that heat-hardening response occurs and to ascertain its time course. To do this, we measured the change in the upper thermal limit (ΔCT) at varying time intervals (1–11 h in 2-h increments) since exposure to the first CT_{max} trial. As well as capturing the heat-hardening response, use of ΔCT as our response variable also mitigates issues associated with measuring air temperature inside the tube rather than core temperature of the animal: individual differences in size and behaviour that might affect the lag between air and core temperature are, if they are constant across the two trials, effectively removed from our response variable. To ensure multiple high-temperature exposures did not influence our results, and to ensure independence, each individual was only tested once. As such, data for different measurement intervals come from different individuals. To check that our results on timing were robust across populations, we ran

these timing experiments for individuals from two populations (Hervey Range [HR] and Paluma, see Fig 1). All these experiments were conducted by a single observer (V. L.).

POPULATION-LEVEL VARIATION

Following the clear result from above that ΔCT was maximized at around 6 h after initial high-temperature exposure (see Results), we made this our standard test interval and proceeded to measure ΔCT across an additional 11 populations of *Lampropholis coggeri*. Although individuals were tested within a fortnight of being collected, it took considerable time to collect all these populations. Consequently, ΔCT across these populations was measured by two observers (A. H.: eight populations, $n = 59$; V. L.: eight populations, $n = 86$; three of these populations were measured by both observers), which we accounted for in our analyses.

STATISTICAL ANALYSES

Site-level temperature data

All analyses were conducted using R (R Core Team 2013). For each of our sampling sites, we extracted the maximum daily under-canopy temperature (DT_{max}) for every day from 1990 to 2009, resolved at 250 m² grid scale (taken from Storlie *et al.* 2013). As expected, DT_{max} showed strong seasonal cycles (Fig. S1). Because we were interested in the predictability of short-term variation in DT_{max} (on the order of days) as well as seasonal variation (on the order of months), we partitioned variance in our data into these two components. To achieve this, we fit a polynomial smoother through the time series data (using a 30-day interval as the smoothing window) and calculated 'short-term' variance as the variance of the residuals around this smoother, and 'seasonal' variance as the total variance minus the short-term variance (See Supporting Information for more details). Because the heat-hardening response we observed had a time course on the order of hours, we also needed a measure of fine-scale predictability. Thus, we calculated 'predictability' as the autocorrelation between successive days of the residual data. In other words, 'predictability' explains how well a given DT_{max} predicted the next day's DT_{max} . These data manipulations gave us three measures associated with DT_{max} for each site: 'seasonal variance'; 'short-term variance'; and 'predictability'. In the analysis, we also added to these the 'mean of DT_{max} ' for each site, calculated over the 20-year time period.

Timing of heat-hardening response

Because we expected heat hardening (ΔCT) to manifest as a temporary increase in the individual's CT_{max} , we fit a quadratic function to the data showing the effect of time on the magnitude of the ΔCT and estimated the time to maximum hardening response. Our data for this analysis were drawn from two populations (Hervey Range and Paluma), so we included population as an additional factor in this model. We assessed the significance of the model coefficients using *t*-tests.

Population-level variation

ΔCT was negatively correlated with initial CT_{max} (see Results). To control for this effect in our population-level analyses, we included initial CT_{max} as a covariate in statistical models. Data on population-level variation across our 13 sampled populations were also collected by two different people (VL and AH), and, consistent with observations in other species and measurement procedures

(e.g. Terblanche *et al.* 2007), there was a considerable observer effect on CT_{max} (see Results). The observer effect on ΔCT was significant through an interaction with the effect of initial CT_{max} (see Results). Accordingly, we included observer (and its interaction with CT_{max}) as a fixed effect in all our analyses of population-level variation. Thus, our inference on the geographic variation in ΔCT and its correlation with environmental factors is made accounting for the effect of observer and initial CT_{max} . To assess whether there was geographic variation in ΔCT , then we used an ANCOVA, with population and observer as fixed effects, and initial CT_{max} (and its interaction with observer) as our covariates.

To assess whether observed geographic variation was correlated with environmental variables (mean DT_{max} , seasonal variance, short-term variance and predictability), we used a linear model relating ΔCT to observer and initial CT_{max} , (plus the interaction between these) as well as the four environmental variables. Our data constituted measurements from 145 lizards, across 13 populations. To correspondingly account for possible pseudoreplication at the population level, we also added population as a random effect in our analysis. This mixed model was performed using the nlme package in R (Pinheiro *et al.* 2013) with output generated by the texreg package (Leifeld 2013).

Results

TIMING AND MAGNITUDE OF HEAT-HARDENING RESPONSE

There was no evidence for an interaction between the two sampled populations and the coefficients of the quadratic response to time ($F_{2,35} = 1.98$, $P = 0.150$), so these interactions were dropped. The resultant quadratic model yielded significant coefficients for the quadratic terms (coefficient of time = 0.62, $t = 3.48$, $P = 0.001$; coefficient of time squared = -0.048 , $t = -3.49$, $P = 0.001$), as well as a marginally significant effect for the population term (effect of Paluma vs. HR = -0.53 , $t = -2.05$, $P = 0.048$). The coefficients for the quadratic imply a peak in heat hardening, ΔCT , occurring at 6.44 h after the initial heat shock (Fig. 2). Following this result, we chose to do all further assays at 6 h post-shock. Across the two populations, in the treatments tested at 5 and 7 h post-heat shock, individual ΔCT ranged from -0.4 to 2.6 degrees.

POPULATION-LEVEL VARIATION

Across all populations and lizards sampled, ΔCT ranged from -0.9 to 2.6 degrees, with a mean of 0.42 degrees (Fig. S1). The ANCOVA exploring variation in ΔCT as a function of population, observer and initial CT_{max} and their interaction (all as fixed effects) revealed significant effects for all independent variables. As discussed above (Materials and methods), there was a significant negative effect of initial CT_{max} ($F_{1,129} = 25.84$, $P < 0.001$; Fig. 3), and a significant interaction between this slope and the observer (Fig 3 $F_{1,129} = 4.87$, $P = 0.029$). There was also a significant observer effect ($F_{1,129} = 53.29$, $P < 0.001$), but given that this significance is assessed at the y-intercept (and there is a clear interaction), this effect is difficult to interpret. Finally, there was also a significant population

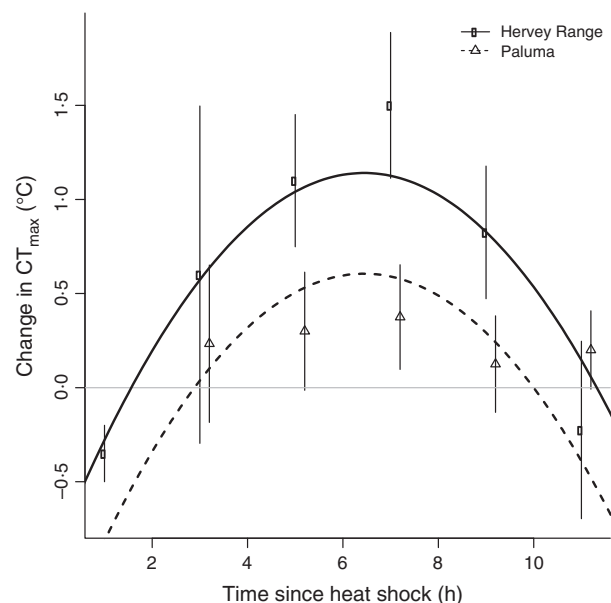


Fig. 2. Heat hardening (change in CT_{max}) as a function of time since the heat shock was given. Two populations were tested, both of which suggested a peak in heat hardening at 6.4 h post-shock. Lines show the fitted model and error bars represent 1 standard error around the mean.

effect ($F_{12,129} = 9.28$, $P < 0.001$), confirming that heat hardening exhibits geographic variation.

How large are these geographic effects, and can variation in hardening, ΔCT , be explained by an environmental driver? Our mixed-effects model confirmed again a substantial and inverse effect of initial CT_{max} on ΔCT (slope estimate = -0.38 , $t = -4.93$, d.f. = 129, $P < 0.001$), in this case without significant observer effects (interaction = -0.11 , $t = -1.11$, d.f. = 129, $P = 0.270$). The analysis also revealed a positive relationship between ΔCT and seasonal variation in DT_{max} (slope estimate = 0.11 , $t = 2.90$, d.f. = 8, $P = 0.019$; Fig. 4), the predictability of DT_{max} (slope estimate = 9.77 , $t = 3.43$, d.f. = 8, $P = 0.0089$; Fig. 4) and mean DT_{max} (slope estimate = 0.047 , $t = 2.61$, d.f. = 8, $P = 0.031$). In contrast, short-term variance in maximum temperature had no effect on ΔCT (slope estimate = -0.10 , $t = -1.24$, d.f. = 8, $P = 0.250$). Correlation coefficients between observer and all other explanatory variables were low (< 0.35) in all cases except for initial CT_{max} , where the correlation coefficient was 0.80 . Comparison of models with and without the environmental terms suggests that local environmental conditions explain effectively 100% of the population-level variance in ΔCT (Table 2).

Discussion

Tropical ectotherms are considered to be particularly vulnerable to climate change because they lack the capacity to regulate their temperature independently of their environment and are thought to be narrowly adapted to the

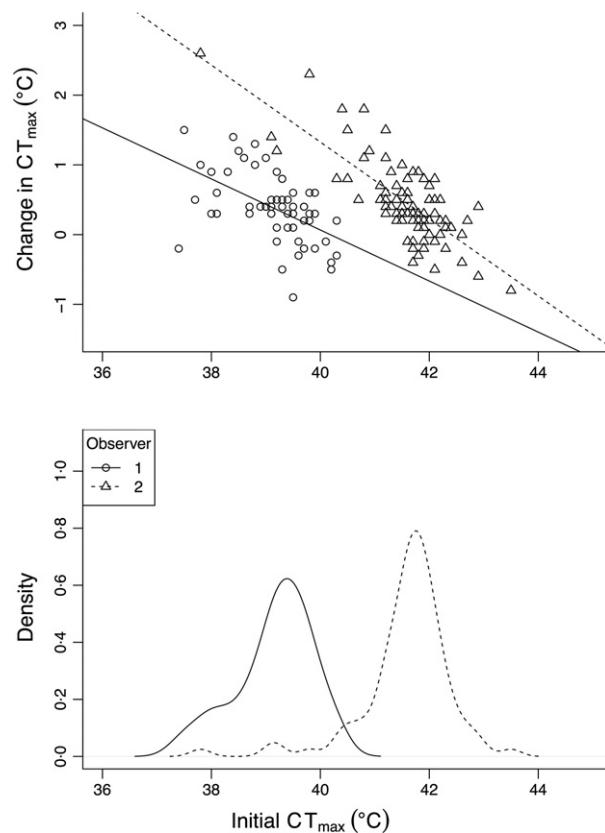


Fig. 3. Observer effects on CT_{max} and ΔCT . The top panel shows how CT_{max} shifted between trials by observer (different shapes). Despite the large difference in CT_{max} between observers, ΔCT did not differ significantly between observers (t -test: $t = 0.88$, d.f. = 143, $P = 0.38$). The bottom panel shows density plots for initial CT_{max} by observer.

relatively stable conditions of low-latitude habitats (Angilletta 2009; Huey *et al.* 2009, 2012). Moreover, because these organisms evolved and persist in habitats with relatively low daily and seasonal temperature variation, they are expected to have relatively poor buffering capacities through physiological acclimation (Brattstrom 1968; Ghalambor *et al.* 2006). Nonetheless, tropical species with distributions across strong climatic gradients may harbour important adaptive variation in physiological traits, and in the plasticity of those traits.

Here, we document just such a plastic response – heat hardening – in the tropical lizard, *Lampropholis coggeri*. When exposed to a single episode of high temperature, individual lizards showed a rapid increase of up to 2.6 °C in their upper thermal limit, with a mean shift in CT_{max} of approximately 0.42 °C (Fig. 3). This hardening response peaked 6–7 h after the initial high-temperature exposure before declining back to baseline levels over an equivalent time period. The timing of this response is somewhat surprising: peak environmental temperatures typically occur in the early afternoon, so the peak in heat hardening in *L. coggeri* will typically occur when the environment is cooling (in the late afternoon or evening). The heat-shock

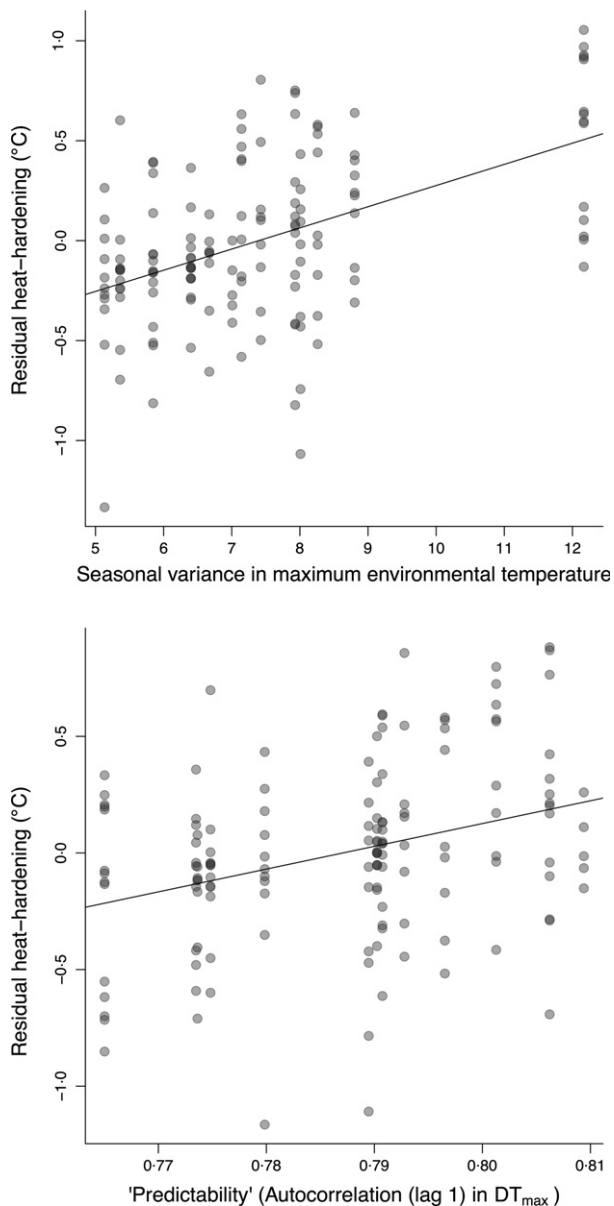


Fig. 4. Heat hardening showed substantial variation between populations, and much of this variation can be explained by a strong relationship between heat hardening and the predictability and seasonality of maximum temperature. The figure panels show heat hardening of animals tested from 13 populations plotted against the predictability and seasonality maximum temperature. The y-axis denotes the residual heat hardening (removing the effect of the other model variables, see Table 2). The plotted line is the slope from the linear model in Table 1 and indicates that the relationship between heat hardening and both seasonal variation and predictability in maximum temperature is positive and significant.

response has manifold effects beyond simply increasing CT_{max} , and these may include increased rates of cellular repair as well as initiating long-term acclimation (Hochachka & Somero 2002; Hoffmann, Sørensen & Loeschcke 2003). As a consequence, it is difficult to determine the reason behind the 12-h cycle of heat hardening that we see here. Nonetheless, the correlations we observe

Table 2. The mixed-effects model exploring effects of site-level environmental variables on the heat-hardening response of *L. coggeri*

	Parameter estimates (SE)
Intercept	5.9566 (3.7420)
Predictability	9.7709 (2.8435)**
Short-term variance in maximum temperature	-0.1033 (0.0834)
Seasonal variance in maximum temperature	0.1060 (0.0365)*
Mean of maximum site temperature	0.0464 (0.0178)*
Initial CT_{max}	-0.3776 (0.0766)***
Observer difference	5.6994 (3.9445)
Interaction: Observer x Initial CT_{max}	-0.1097 (0.0988)
Log Likelihood	-57.9595
Number of observations	145
Number of groups	13

Variables that significantly contributed to the final model are highlighted in bold, with significance denoted as follows: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Variance explained by population in this model was effectively zero (precisely, 1.2×10^{-10}), whereas a model without the fixed environmental effects had substantial population-level variance (2.7×10^{-2}).

between hardening and the lizards' thermal environment (discussed below) suggest that the heat-hardening response we elicited in the laboratory involves physiological machinery that has been optimized by climate-imposed natural selection.

The strength of the heat-hardening response was strongly contingent on initial thermal tolerance, such that individuals with a relatively high initial CT_{max} showed a relatively small heat-hardening response compared to lizards with lower initial CT_{max} . This inverse relationship between thermal limits and hardening response is commonly observed in *Drosophila* (e.g. Berrigan & Hoffmann 1998; Sørensen, Dahlggaard & Loeschcke 2001; Zatschina *et al.* 2001), and, across species, has also been shown in crabs (Stillman 2003). Its presence also in our lizard suggests a general overlapping biochemical basis to the two traits (as has been pointed out by Somero 2002; Bowler 2005). It is noteworthy that, in *L. coggeri*, field variation in CT_{max} (varying between 36.5 and 41.5 °C) appears to be driven largely by acclimation to recent daily maximum temperatures (DT_{max}) experienced in the lizards' environment (J. Llewelyn, S. Macdonald, A. Hatcher, C. Moritz, B. L. Phillips, unpublished data). Thus, our results suggest that the magnitude of the heat-shock response in *L. coggeri* depends on its field acclimation temperature, and this implies a hard upper limit to this species' capacity to adjust its upper thermal tolerance, whether through acclimation or hardening. In individuals, already acclimated to high temperatures, the capacity to mount a further heat-hardening response is severely compromised.

The strength of this heat-hardening response also varied between populations: in some populations the heat-hardening response was weak to non-existent, whereas in others

it was substantial. Moreover, this geographic variation in heat hardening was strongly associated with local maximum temperatures, particularly with respect to the seasonality and predictability of DT_{max} . The clearest result from our analysis of the lizards' environment affirms expectations that heat hardening is only beneficial in predictably variable habitats (Gilchrist 1995; Kingsolver & Huey 1998). Although DT_{max} was, overall, highly predictable on a day-to-day basis (i.e. autocorrelation coefficients ranging from 0.76 to 0.81), that small amount of variation in predictability between sites accounted for a large portion of the population-level variance in heat hardening (Fig. 4).

Whereas our results regarding environmental predictability are in clear agreement with theory, the expectation that hardening capacity should also scale with short-term fluctuation in DT_{max} was not met. Instead, we found that seasonal variability explained considerably more variation in heat hardening. Given the time-scale of the heat-hardening response (order of hours), this result is somewhat perplexing. Why would seasonal variation matter for such a short-term response? We suspect the answer lies in a biochemical overlap between the short-term heat-hardening response and the longer term acclimation response. Acclimation involves a suite of biochemical changes both in the number and type of proteins expressed, as well as changes to the lipid composition of cell membranes (reviewed in Bowler 2005; Angilletta 2009). Heat hardening, in contrast, is associated primarily with the increased production of heat-shock proteins, the production of which is triggered by the accumulation of damaged proteins in cells (reviewed in Hoffmann & Parsons 1991; Somero 2002; Bowler 2005). Heat-shock proteins are associated with both acclimation and hardening (Somero 2002; and references therein), and hardening can have a cumulative effect (repeated exposure to high temperature cumulatively increases thermal tolerance, Hoffmann, Sørensen & Loeschcke 2003 and references therein). Thus, repeated heat shock begins to have effects that look like acclimation, suggesting that the two phenomena are mechanistically linked. It seems quite possible, then, that selection for greater acclimation ability (on the scale of weeks or months) may also provide an increased capacity to mount rapid high-temperature-hardening responses.

The trait–environment association we uncover here is of more than theoretical interest. Climate change is already well underway, and there is justifiable concern that lowland tropical forest-dwelling species – with their narrow thermal niches – may be particularly hard hit (Huey *et al.* 2009, 2012). Although tropical species may have narrower thermal limits, it may be that because of this they exhibit closer adaptation to local climatic conditions. When coupled with low dispersal rates, as in this species (Singhal & Moritz 2012), much of the capacity for tropical species to adapt to climate change may be found across space rather than within populations or individuals. Our results are consistent with this possibility, with clear patterns of local

adaptation in heat hardening emerging over quite small spatial scales. This high trait turnover raises the possibility that gene flow – occurring either naturally or as part of an assisted gene flow strategy (Aitken & Whitlock 2013) – can assist populations of tropical species adapt to climate change. Our work also highlights, however, that there may be hard upper limits that ultimately constrain how high CT_{max} can shift. This implies a temperature threshold beyond which we would not expect the species to adapt. Nonetheless, as extreme events become more common into the future, locating populations pre-adapted to that new regime may be of critical importance, and environment–trait associations such as the ones we have uncovered here will be invaluable to that effort.

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Data accessibility

The data and scripts are stored at FigShare (DOI: 10.6084/m9-fgshare.1566871)

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Raw and detrended data on maximum temperature at one of our sites (Paluma, 5220A).

Table S1. Pairwise distance (in kilometres) between our survey sites.