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Geographic Variation in the Effects of Heat Exposure on Maximum Sprint Speed and Hsp70 Expression in the Western Fence Lizard *Sceloporus occidentalis*

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ABSTRACT

We examined whether western fence lizards *Sceloporus occidentalis* occurring in thermally divergent environments display differential responses to high temperature in locomotor performance and heat-shock protein (Hsp) expression. We measured maximum sprint speed in *S. occidentalis* from four populations at paired latitudes and elevations before and after exposure to an experimental heat treatment and then quantified hind-limb muscle Hsp70 expression. Lizards collected from northern or high-elevation collection sites suffered a greater reduction in sprint speed after heat exposure than lizards collected from southern or low-elevation sites. In addition, lizards from northern collection sites also exhibited an increase in Hsp70 expression after heat exposure, whereas there was no effect of heat exposure on Hsp70 expression in lizards from southern collection sites. Across all groups, there was a negative relationship between Hsp70 expression and sprint speed after thermal stress. This result is significant because (a) it suggests that an increase in Hsp70 alone cannot compensate for the immediate negative effects of high-temperature exposure on sprint speed and (b) it demonstrates a novel correlation between an emergent property at the intersection of several physiological systems (locomotion) and a cellular response (Hsp70 expression). Ultimately, geographic variation in the effects of heat on sprint speed may translate into differential fitness and popu-

lation viability during future increases in global air temperatures.

Introduction

Environmental temperature varies greatly across geographic regions, and it is known to limit species distributions through its effects on reproduction and survival in many taxa (Jenkins and Hoffmann 1999; Parmesan et al. 1999; Helmuth and Hofmann 2001). As a consequence, changes in temperature over geological and ecological timescales have been linked to range shifts as well as local extinctions of species (Parmesan et al. 1999; Clarke 2003; Rank et al. 2007). Understanding the link between temperature and the distribution of species or populations is especially relevant given that current models of climate change predict that average global temperature will increase by a minimum of 0.15°C per decade, with greater increases at higher latitudes and elevations, and an increase in the frequency and intensity of extreme temperatures (Jayko and Millar 2001; Walther et al. 2002; IPCC 2007). These predicted changes in temperature may be especially important for ectothermic animals for which variation in ambient temperature (T_a) may have especially pronounced effects on whole-animal performance capacities, survival, and geographic distribution (Helmuth et al. 2002; Pörtner 2002; Parmesan 2006; Deutsch et al. 2008).

Whole-organism performance traits arise from interactions among morphology, behavior, and physiology, often as a result of strong natural and sexual selection (Arnold 1983; Irschick et al. 2008). Maximum sprint speed is an example of an ecologically relevant measure of whole-organism performance that is often important for eluding predators and capturing prey (Bennett 1990; Garland and Losos 1994) and has been extensively studied in lizards (Irschick et al. 2008). As with many whole-organism performance traits, sprint speed typically shows an asymmetric bell-shaped curve in which performance is poor at low T_a 's, increasing to a plateau of higher performance at moderate temperatures, and followed by a precipitous decline at very high temperatures (Huey and Kingsolver 1993). If lizards cannot sprint at or near their maximum capacities, then this could effect many other traits, such as their ability to elude predators (de Barros et al. 2010; Okafor 2010). As a consequence, reduction in sprint speed at nonoptimal body temperatures (T_b 's) is potentially coupled with negative effects on

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fitness. Indeed, a recent review examining the relationships between whole-organism performance traits and fitness revealed that in about half the cases, animals with high-performance capacities were favored by natural selection (Irschick et al. 2008).

Another effect of high temperatures is that cellular proteins become denatured because of the perturbation of weak interactions stabilizing protein structure and function (Hofmann and Somero 1996; Hochachka and Somero 2002). In many organisms, this leads to increased expression of molecular chaperones called heat-shock proteins (Hsps), which prevent further denaturation and help restore function to damaged proteins (Parsell and Lindquist 1993; Feder and Hofmann 1999; Sorensen et al. 2003). The expression of inducible Hsp isoforms after thermal stress supplements constitutively expressed Hsps already present in cells that assist in folding newly translated proteins (Morimoto et al. 1994; Hochachka and Somero 2002). Increased levels of Hsps, specifically Hsp70-class chaperones (68–76 kDa), are correlated with increased thermal tolerance and survival in a wide variety of organisms, including fish (Fader et al. 1994; Fangue et al. 2011), reptiles (Ulmasov et al. 1992), and invertebrates (Hofmann and Somero 1995; Yocum 2001; Rinehart et al. 2007). However, the overproduction of Hsps is also known to incur a substantial physiological cost (Krebs and Holbrook 2001; Sorensen et al. 2003), although this relationship is still being tested in natural populations (Tomanek and Somero 2000; Rank et al. 2007).

In this study, we examined the simultaneous effects of heat treatment on one measure of organismal performance (maximum sprint speed) and one indicator of cellular stress (muscle Hsp70 expression) in the western fence lizard *Sceloporus occidentalis* collected from field sites that vary in their thermal regimes. We predicted that exposure to elevated temperatures would more adversely affect both metrics in animals from cooler habitats compared with those from warmer habitats. *Sceloporus occidentalis* is ideal for studies of thermal physiology for several reasons: (1) it is widely distributed in thermally diverse habitats throughout western North America, (2) sprint speed varies among populations collected in different parts of its range (Buckley et al. 2007), and (3) the relationship between temperature and sprint speed follows the typical trend described earlier in which sprint speed increases to about 35°C and then declines sharply above that temperature (Adolph 1987; Angilletta et al. 2002). Our study extends previous work on the thermal effects on locomotion by incorporating Hsp expression to assess the relationship between locomotion and this indicator of cellular stress. In addition, our study expands on a growing body of literature that examines geographic variation in physiological performance and the cellular stress response (Lenormand 2002; Karl et al. 2009). Geographic variation in the effects of elevated temperatures on thermal physiology may provide insight into how populations of ectothermic animals will respond to increased T_a during global climate change (Somero 2011).

Methods

Study Sites and Animals

The western fence lizard *Sceloporus occidentalis* occurs throughout western North America from Washington State to central Baja California, Mexico, and from sea level to approximately 2,500 m (Stebbins 2003; Buckley et al. 2010; Fig. 1a). Adults average 60–90 mm snout to vent and are semi-arboreal habitat generalists, utilizing perches consisting of boulders, bushes, and trees. This species is typically active at the sites used for this study from April to October (Asbury and Adolph 2007). All lizards used here were collected from four locations in California: Bishop Creek (BC), 7.9 km southwest of Bishop, Inyo County (37°17'34"N, 118°33'05"W, elevation 2,135 m); Keough Hot Springs (KHS), 12.5 km south of Bishop, Inyo County (37°15'4"N, 118°22'20"W, elevation 1,220 m); Table Mountain (TM), 3.5 km northwest of Wrightwood, San Bernardino County (34°22'43"N, 117°39'33"W, elevation 2,230 m); and Largo Vista (LV), 11.2 km west of Pinion Hills, Los Angeles County (34°26'30"N, 117°45'42"W, elevation 1,370 m). These four sites represent two geographic regions (north and south) with paired high and low elevations. Previous work has shown that lizards from these two geographic regions are genetically distinct from one another (S. Adolph, unpublished data), although there are no clear data showing genetic divergence between high and low elevations within either the northern locations or the southern locations.

In order to characterize the thermal habitat at each site, environmental temperature was measured using Pendant data loggers (UA-002-64, Onset Computer, Pocasset, MA) suspended in inverted opaque white plastic cups with holes cut to allow for ventilation. Loggers were affixed to randomly chosen trees at each site and programmed to record T_a every 20 min between April and September of each year beginning August 20, 2005, and ending September 20, 2008. Measurement of air temperature at one site (TM) was not possible between June 15, 2007, and June 25, 2008, because the logger was destroyed in a brush fire.

At each site, 13–15 adult male lizards were collected between May and September 2007, with an additional six lizards collected between May and September 2008. All lizards were transported to the laboratory within 8 h of capture and housed in custom-made 25-L wooden enclosures with screen tops. A single rock was included for a perch, and sand was used as a substrate. Heat lamps suspended above each cage provided a 12L : 12D cycle and a thermal gradient that allowed lizards to thermoregulate. Water was provided ad lib. in a small dish; crickets dusted with vitamin powder were provided as food every other day. The presence of food in the stomach has been shown to affect locomotor performance in other species (Kelehear and Webb 2006), so lizards were not fed within 24 h before experiments. All research conducted herein conformed to national and institutional guidelines for research on vertebrate animals (IACUC 27-10-02, University of Massachusetts, Amherst).

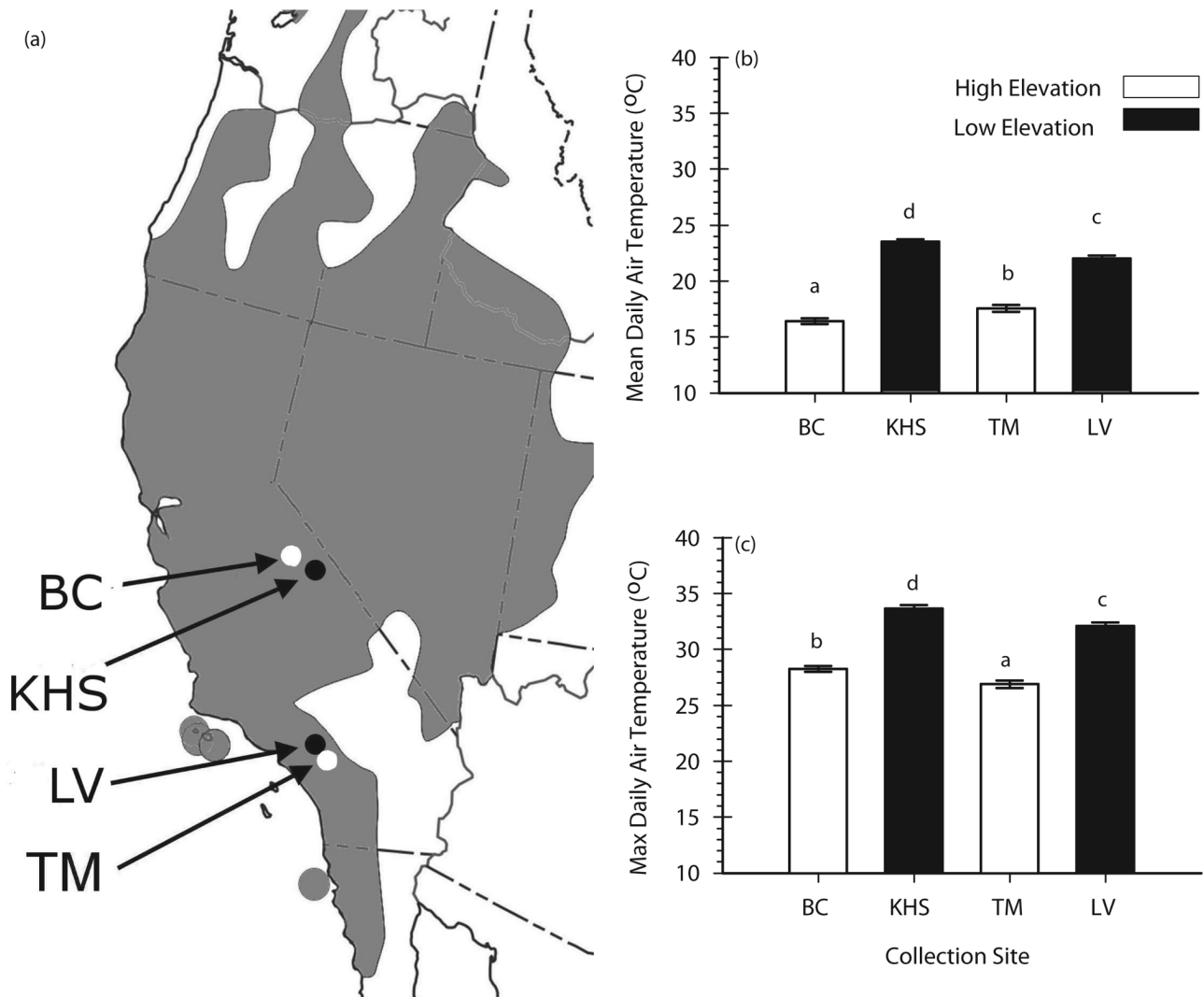


Figure 1. Geographic distribution of the western fence lizard *Sceloporus occidentalis* and collection site temperatures from April to September 2005–2008. *a* shows the distribution of *S. occidentalis* (shaded region) and the location of study sites: Bishop Creek (BC), Keough Hot Springs (KHS), Table Mountain (TM), and Largo Vista (LV). Distribution map adapted from Buckley et. al. (2010). *b* shows daily mean temperature and *c* shows daily maximum temperature at the four collection sites (\pm SEM). See Table A1 in the online edition of *Physiological and Biochemical Zoology* and text for statistical analysis.

Heat Treatment and Sprint Speed Trials

Sprinting performance trials were conducted within 72 h of capture. Sprint speed was measured at 22°C using a custom-built 2-m racetrack with photo gates every 25 cm. The time required to traverse each 25-cm interval was converted into measurements of speed, and the interval with the highest speed was recorded as the sprint speed for that trial. Three trials were conducted for each lizard during each sampling period, and a mean maximum speed was calculated using the fastest interval during each of the three trials. To minimize the change in T_b during sprint speed measurement, each lizard was measured immediately on removal from the incubator and returned to

the environmental chamber while other individuals were measured.

To test for differential responses in sprint speed and Hsp70 accumulation in response to elevated temperatures, lizards were randomly assigned to one of two experimental treatments: a heat treatment at 40°C ($N = 14$ –16 per site) or a control treatment at 22°C ($N = 5$ per site). Sprint speed was measured in all lizards before exposure to experimental treatment (0 h, time point T1). After initial sprinting trials, each lizard was placed in a small plastic container in a temperature incubator at either 40°C (heat treatment) or 22°C (control) for 4 h. At the end of the experimental treatment, sprint speed was measured in all lizards (4 h, time point T2). Then all lizards were held for 1

Table 1: Mean sprint speed and body temperature (T_b) for *Sceloporus occidentalis* collected from four collection sites and exposed to heat or control treatments

	T1 (0 h)	T2 (4 h)	T3 (5 h)
Sprint speed (m/s):			
BC	1.537 (.110) ^A	.709 (.070) ^B	.693 (.930) ^B
KHS	1.576 (.076) ^A	1.326 (.188) ^A	1.020 (.131) ^B
TM	1.326 (.095) ^A	1.131 (.142) ^B	.934 (.116) ^C
LV	1.821 (.146) ^A	1.924 (.218) ^{A,B}	1.652 (.147) ^B
Pooled controls	1.375 (.094) ^A	1.185 (.134) ^A	1.222 (.094) ^A
T_b (°C):			
BC	23.39 (.13) ^A	37.24 (.18) ^C	24.93 (.14) ^B
KHS	23.34 (.15) ^A	37.03 (.17) ^C	24.85 (.19) ^B
TM	23.84 (.21) ^A	37.55 (.23) ^C	24.32 (.17) ^B
LV	23.88 (.12) ^A	37.06 (.12) ^B	24.05 (.17) ^A
Pooled controls	22.59 (.10) ^A	24.27 (.67) ^C	23.23 (.09) ^B

Note. Values represent means (\pm SEM) of each site for lizards exposed to experimental heat treatment (40°C) for 4 h or means for lizards from all four sites exposed to control temperature treatment (22°C) for 4 h. Letters denote significant statistical differences between time points within a single group (site or pooled controls). Statistical significance was determined using matched paired *t*-tests for every possible combination of time points (T1 vs. T2, T1 vs. T3, and T2 vs. T3). BC = Bishop Creek; KHS = Keough Hot Springs; TM = Table Mountain; LV = Largo Vista.

h at 22°C to allow for the synthesis of Hsps (Dahlhoff et al. 2001; McMillan et al. 2005), and sprint speeds were measured again (5 h, time point T3). Sprint speed is highly dependent on T_b in iguanid lizards (Huey and Kingsolver 1993; Du et al. 2007; Okafor 2010). Accordingly, T_b was measured immediately on completion of each sprint replicate using a cloacal thermometer, and mean T_b was calculated for each individual at each experimental time point.

After completion of the sprint speed and T_b measurements, all lizards were euthanized by decapitation. Upper hind-limb muscle tissue was dissected, including the triceps femoris and sartorius as well as the gracilis major and gracilis minor muscles, all of which are made up of fast-twitch glycolytic muscle fibers and are presumed to be used for power-burst locomotion activities in iguanid lizards (Gleeson and Harrison 1986). After dissection, all tissues were frozen on dry ice and stored at -80°C.

Biochemical Analyses

Levels of Hsp70 were quantified following methods modified from previous studies (McMillan et al. 2005). Dissected tissues were homogenized in approximately 10 volumes of buffer (100 mM Tris-HCl, 0.5% SDS, 0.5% Triton-X, 1% Protease inhibitor cocktail [Sigma-Aldrich], pH 7.2) using ground-glass tissue homogenizers (Kontes Duall 20) and centrifuged at 15,000 *g* for 15 min at 4°C. Total protein concentration was determined in the supernatant using a commercially available kit (Thermo Fisher Scientific, Rockford, IL) following a modified bicinchoninic acid assay (Smith et al. 1985). Samples of 20 μ g total protein were reduced with 50 mM dithiothreitol combined with SDS-sample buffer (Invitrogen, Carlsbad, CA) and heated at

95°C for 3–5 min to ensure complete protein denaturation. Samples were then separated by SDS-polyacrylamide gel electrophoresis (Laemmli 1970) on 4%–12% gradient gels using commercially available MOPS running buffer (Invitrogen). After electrophoresis, proteins were transferred to 0.45 μ m PVDF membranes using a constant current (400 mA) according to published methods (Towbin et al. 1979; McMillan et al. 2005). Blots were then probed for Hsp70 using an Hsp70-specific antibody (MA3-006, Affinity BioReagents, Golden, CO) and a peroxidase conjugated antimouse secondary antibody. Blots were developed using chemiluminescence, images were captured by a CCD camera (Bio-Rad Chemi-Doc), and band intensities were quantified with Quantity One software (Bio-Rad). Relative quantification of Hsp70 expression was achieved by loading two wells of each gel with human Hsp70 (NSP-555; Assay Designs, Ann Arbor, MI) as positive controls. The amount of standard ranged from 10 to 80 ng per well. To standardize protein quantification across gels, all gels shared one quantity of the positive control with at least one other gel. All Hsp70 values reported here represent the band intensity relative to the overall mean Hsp70 expression of lizards in the control group. Importantly, the primary antibody used is capable of detecting multiple Hsps from ~70 to ~78 kDa, which includes both inducible and constitutive members of the Hsp70 family. Therefore, the measures of Hsp70 concentration reported in this study reflect the sum of these forms.

Statistical Analyses

To evaluate differences in habitat temperatures, we calculated daily mean temperature and daily maximum temperature at each site and included them as dependent variables in two-way

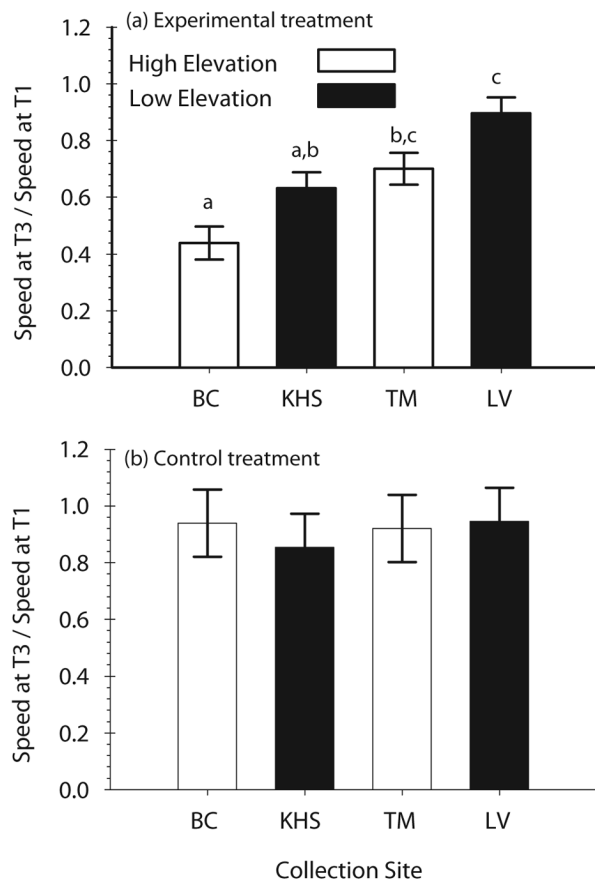


Figure 2. Effects of experimental treatment on sprint speed performance in *Sceloporus occidentalis*. Bars represent the proportion of sprint speed (T3/T1) remaining after heat (a) or control (b) treatments. Heat treatment consisted of 4-h exposure to 40°C followed by 1 h at 22°C, while control treatment consisted of 5 h at 22°C. Bars with different letters were significantly different, and error bars represent 1 SEM. See Table A2 in the online edition of *Physiological and Biochemical Zoology* and text for statistical analysis.

ANOVAs with collection site region (north or south) and elevation (high or low) as main factors. Preliminary analyses of sprint speed demonstrated that speeds measured at room temperature (22°C) without previous laboratory heat treatment did not differ significantly between collection years ($F = 0.9848$, $df = 1, 78$, $P = 0.3241$) nor were they significantly related to snout-vent length ($F = 0.1547$, $df = 1, 78$, $P = 0.6951$). Accordingly, sprint speed data were pooled from both years, and snout-vent length was excluded from all further analyses. Because initial sprint speeds varied among collection sites (Table 1), sprint speed remaining at the end of the experiment (T3) was expressed relative to initial sprint speed (T1) for each individual, and the effects of region and elevation on this proportion (T3/T1) were assessed with a full-factorial two-way ANOVA. To evaluate variation in Hsp70 expression, we used a three-way ANOVA, with region, elevation, and experimental treatment (heat or control) as main effects (including all in-

teractions). Finally, to examine the relationship between Hsp70 expression and posttreatment sprint performance, a two-way ANCOVA was used, with the proportion of sprint speed retained after heat treatment (T3/T1) as the dependent variable, region and elevation as main effects, and Hsp70 expression as a covariate. All interactions were included in this model to test for heterogeneity of slopes among sites in the relationship between Hsp70 expression and proportion of sprint speed retained. Post hoc pairwise comparisons for all analyses were made with Tukey's HSD tests; P values ≤ 0.05 were taken to indicate statistical significance. All tests were conducted using JMP statistical software (ver. 9.0.0; SAS Institute, Cary, NC).

Results

Habitat Temperatures

Ambient air temperature was measured at the four collection sites from August 2005 to September 2008. Measurements of daily mean and daily maximum air temperatures during the spring and summer months (April to September) showed that the sites varied significantly (Fig. 1; see Table A1 in the online edition of *Physiological and Biochemical Zoology*). As expected, both measurements of temperature were strongly influenced by elevation, with low-elevation sites being significantly warmer than high-elevation sites ($>5^{\circ}\text{C}$ difference on average; $P < 0.0001$ for mean and maximum temperature; Table A1). There was also a significant difference in daily maximum temperatures between geographic regions (north and south; Fig. 1; Table A1; $P < 0.0001$), although this effect was generally smaller than the effect of elevation ($<1.5^{\circ}\text{C}$ on average) and not necessarily in the direction predicted for a latitudinal effect. During the measurement period (581 d total), we also noted the number of days in which the maximum temperature exceeded 40°C as an index of potentially stressful temperatures for these lizards. This measurement paralleled the trends observed in daily maximum temperature, with the greatest number of days above 40°C at the northerly low-elevation site (71) followed by the southerly low-elevation site (38), the northerly high-elevation site (8), and the southerly high-elevation site (3).

Sprint Trials

Maximum sprint speed measured at 22°C before the experimental or control treatments (T1) was significantly higher for lizards collected at low-elevation sites (Table 1; $P = 0.0204$). When subjected to a 4-h treatment at 40°C followed by a 1-h recovery, maximum sprint speeds decreased in lizards collected at all sites. In all cases, sprint speeds were greatest at the beginning of the experiment (T1) and lowest when measured at the end of the recovery period (T3), with sprint speeds measured immediately after heat exposure (T2) generally being intermediate (Table 1). The exception was at BC, for which sprint speeds decreased significantly between T1 and T2 and did not decrease further from T2 to T3 (Table 1). Lizards in the control treatment ($N = 5$ from each site) were handled in an identical manner to experimental lizards except that they

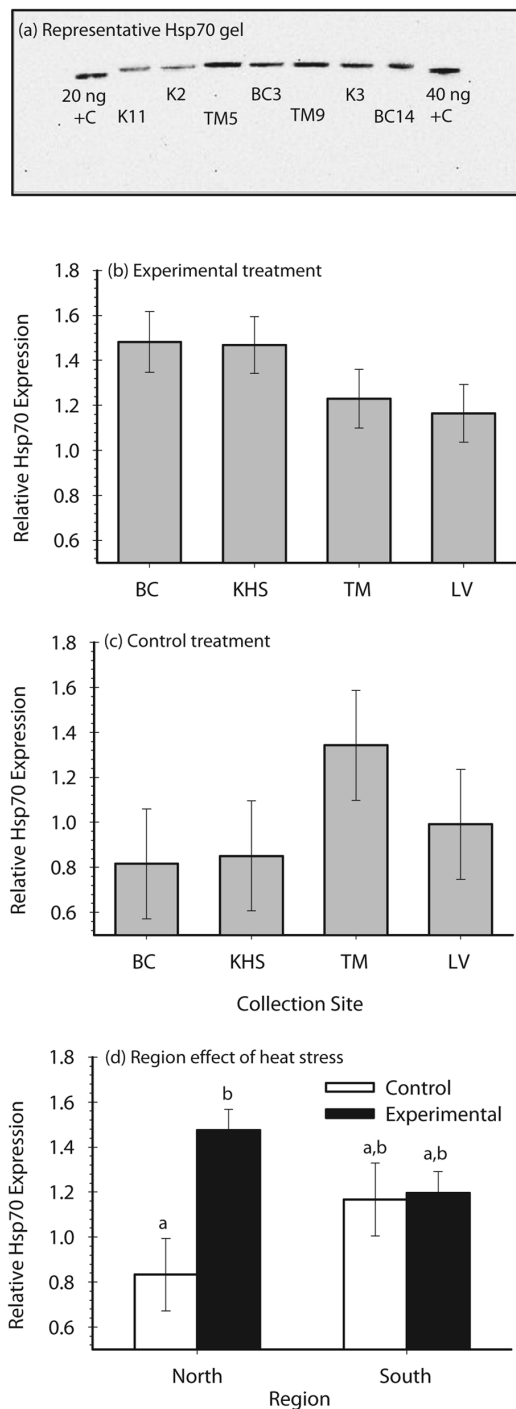


Figure 3. Hsp70 expression in *Sceloporus occidentalis* from differing regions and elevations of collection. *a* shows a representative Hsp70 western blot. Lanes 2–9 contain hind-limb muscle samples from different lizards; lanes 1 and 10 contain known amounts of human Hsp70. *b* and *c* show relative Hsp70 expression (\pm SEM) for heat-treated and control lizards, respectively. Hsp70 expression was calculated relative to the mean Hsp70 expression for all control lizards. *d* illustrates the interaction between treatment and region of collection in relative expression of Hsp70. Letters denote significance differences between groups. See Table A3 in the online edition of *Physiological and Biochemical Zoology* and the text for statistical analysis.

were held at 22°C for 4 h in place of the heat treatment. There were no differences in sprint speeds among T1, T2, and T3 for control lizards, indicating that the procedures and handling did not adversely affect sprint speed independent of the heat treatment (Table 1). Lizard T_b varied little within sites between T1 and T3 when lizards had been held at 22°C. However, T_b did vary among sites at T1 and T3 (Table 1; $P = 0.0283$ and 0.0011 for T1 and T3, respectively). Using post hoc tests, at T1 we did not find specific differences among sites, yet at T3, BC and KHS lizards had a higher T_b after the recovery period than TM and LV lizards. While statistically significant, this difference in T_b was less than 1°C.

Because starting sprint speeds (T1) differed according to elevation (see above), we expressed the change in sprint speed after experimental treatment as the sprint speed at T3 divided by the sprint speed at T1. In addition, T_b was similar in lizards at T1 and T3, removing the potentially confounding effect of variable T_b on maximum sprint speed. The ratio of speeds at T3/T1 was then analyzed for variation due to collection site region and elevation (Fig. 2; see Table A2 in the online edition of *Physiological and Biochemical Zoology*). For lizards subjected to 40°C for 4 h followed by 1 h of recovery at 22°C, the proportion of speed remaining at the end of the experiment varied from about 0.45 to 0.90, and this proportion was strongly affected by both region ($P < 0.0001$) and elevation ($P = 0.0011$); maximum sprint speed was more dramatically reduced by heat treatment in lizards collected from northern or high-elevation sites compared with southern or low-elevation sites (Fig. 2*a*; Table A2). Lizards that were not subjected to the 40°C heat treatment (controls) had maximum sprint speeds at T3 that were 0.915 ± 0.055 of sprint speeds at T1 (mean \pm 1 SEM, $N = 20$); furthermore, speeds did not vary among control lizards from the four study populations (Fig. 2*b*; Table A2).

Hsp70 Expression and Correlation with Sprint Speed Reduction

Quantitative western immunoblotting of total Hsp70 protein expression (constitutive + inducible isoforms) from hind-limb muscle revealed considerable variability (Fig. 3*a*). When heat-treated and control animals were analyzed separately, there were no differences in Hsp70 expression among sites (Fig. 3*b*, 3*c*; see Table A3 in the online edition of *Physiological and Biochemical Zoology*). However, when Hsp70 expressions in heat-treated and control lizards were analyzed together, there was a significant effect of treatment (heat vs. control) on Hsp70 expression (Table A3; $P = 0.0131$) and a significant interaction between treatment and region of collection on Hsp70 expression (Table A3; Fig. 3*d*; $P = 0.0234$). The effect of heat treatment on total Hsp70 expression differed by region. For lizards collected from the two northern sites, the mean Hsp70 expression was nearly twice as high in heat-treated lizards as in control lizards. For the southern collection sites, however, Hsp70 protein expression was intermediate in concentration and did not differ between heat-treated lizards and control lizards.

The observation of geographic variation in the effects of heat

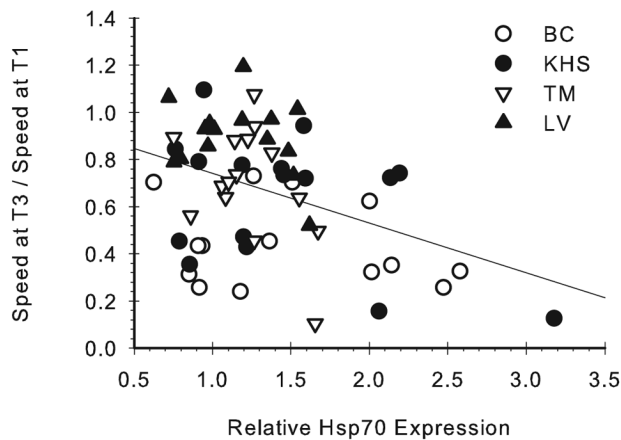


Figure 4. Correlation between Hsp70 relative expression and the proportion of sprint speed retained (T3/T1) for *Sceloporus occidentalis* lizards exposed to 4-h heat treatment and 1-h recovery. Data points represent individual lizards from Bishop Creek (open circles; northern, high elevation; $N = 14$), Keough Hot Springs (filled circles; northern, low elevation; $N = 16$), Table Mountain (open triangles; southern, high elevation; $N = 15$), and Largo Vista (filled triangles; southern, low elevation; $N = 15$). The line is the least squares means line for data from all lizards ($r^2 = 0.1607$, $P = 0.0139$). See Table A4 in the online edition of *Physiological and Biochemical Zoology* and text for statistical analysis.

treatment on both maximum sprint speed and muscle Hsp70 expression suggests that these two variables might be correlated. Accordingly, we tested for covariation between the proportion of sprint speed remaining at the end of the experiment (T3/T1) and Hsp70 expression for lizards from all four collection sites. Because there was not significant heterogeneity among the slopes of T3/T1 versus Hsp70 expression (see Table A4 in the online edition of *Physiological and Biochemical Zoology*; $P = 0.2554$), we pooled data from the four collection sites and plotted T3/T1 against Hsp70 expression (Fig. 4). When thus analyzed, there was a significant negative correlation between the proportion of sprint speed remaining at T3 and Hsp70 expression ($r^2 = 0.1607$, $P = 0.0139$).

Discussion

In this study, we measured the effects of heat exposure on maximum sprint speed and Hsp70 expression in the western fence lizard *Sceloporus occidentalis* collected from high- and low-elevation sites at two geographic regions. We refer to these regions as northern and southern, although we recognize that the north-south distribution of this species is approximately four times greater than the distance between our field sites (Buckley et al. 2010; Fig. 1a). Perhaps because of the limited distance between our northern and southern regions, air temperatures during the summer field season were similar in terms of both daily mean temperature and daily maximum temper-

ature. In contrast, air temperatures were strongly affected by elevation of collection site in both the northern and southern regions: low-elevation sites were significantly warmer than high-elevation sites. The difference in daily mean temperature or daily maximum temperature between low- and high-elevation sites ranged from 5.5° to 8.5°C for sites that differed by approximately 900 m in elevation, which is comparable with the standard lapse rate of 6.5°C/1,000 m (Jacobson 2005). The number of high-temperature days ($\geq 40^\circ\text{C}$) was also greater at the low-elevation collection sites. Taken together, these indicators of mean and extreme temperatures demonstrate that elevation is more important than regional differences in determining the thermal habitat of *S. occidentalis* across the sampled portion of this species' distribution.

Exposure of lizards to a temperature near or above the upper temperatures encountered in the field (40°C for 4 h) caused significant reduction in maximum sprint speed. In the most adversely affected lizards, sprint speeds after heat exposure were reduced to less than half those of the speeds before heat treatment. Interestingly, the extent of this reduction differed among lizards from the different collection sites, with both region and elevation being significantly related to the loss of sprint performance. Typically, geographic variation in physiological function within a species has been attributed to either acclimatization or local adaptation, both of which enhance performance under particular environmental conditions (Endler 1977; Hochachka and Somero 2002). Because air temperatures during the months before and during animal collection were similar across geographic regions, acclimatization to habitat temperature would not appear to account for the regional effect on sprint speed reduction. Rather, the regional effect could be due to some other habitat variable (e.g., availability of prey or predators), long-lasting developmental effects, or genetic differences across this portion of the species range (S. Adolph, unpublished data). On the other hand, acclimatization may play a role in determining the effects of elevation on sprint speed reduction because habitat temperatures at the low-elevation sites were significantly warmer than the high-elevation sites and exceeded our experimental heat treatment several times within the collection season.

In addition to measures of maximum sprint speed, we also found geographic variation in the effects of temperature on Hsp70 expression in hind-limb muscles. Specifically, heat treatment caused an increase in muscle Hsp70 levels in lizards collected from the northern sites, while there was no change in lizards from southern sites. Although our measurements do not distinguish between constitutive and inducible Hsp70, this finding suggests that lizards from the northern regions may have a lower onset temperature for inducible isoforms of Hsp70. In this scenario, the 40°C heat treatment would have exceeded the induction temperature in northern lizards but not in southern ones. Geographic variation in Hsp70 induction temperature has been previously shown in intertidal mollusks (Tomanek and Somero 1999; Dutton and Hofmann 2009), insects (Dahlhoff and Rank 2000), amphibians (Sorensen et al. 2009), and reptiles (Ulmasov et al. 1992). With minimal regional effects

on air temperature in our study, the difference in the Hsp70 response between northern and southern collections of *S. occidentalis* can possibly be explained by long-lasting developmental effects or local adaptation. Firm conclusions regarding the relative contribution of short-term and long-term processes in explaining the patterns of high-temperature effects on sprint speed and Hsp70 expression in *S. occidentalis* will require broader sampling combined with controlled laboratory exposures. Notwithstanding, the important observation here is that we documented variation in both variables despite limited geographic sampling.

While a large body of research has examined the temperature effects on ectotherm physiology, we are aware of no previous studies that have simultaneously measured locomotor performance and Hsp expression after heat exposure in a vertebrate species. Thus, a novel finding of this study is the negative relationship between sprint speed and levels of Hsp70 after exposure of lizards to high temperature. Albeit correlative, the linear relationship we present suggests that 16% of the variation in sprint speed after heat stress can be explained by variation in Hsp70 expression. Given the integrative nature of sprint performance, which requires several physiological systems (neural, cardiovascular, muscular), it is perhaps surprising that the correlation with a single index of the cellular response to heat is this high. The negative relationship between Hsp70 expression and maximum sprint speed can be interpreted several ways. Perhaps the most straightforward explanation is that high temperature directly damaged the proteins that make up the contractile apparatus of skeletal muscle, simultaneously lowering the lizard's ability to sprint and promoting the synthesis of Hsps. Another explanation is that both sprint performance and Hsp70 expression are independently related to other physiological, biochemical, or genetic characteristics of the organism (Haag et al. 2005; Rank et al. 2007). Regardless of the possible mechanisms underlying the correlation between sprint speed and Hsp70 expression after high-temperature exposure, a critical observation is that the correlation is negative rather than positive. Our data do not support the hypothesis that greater Hsp70 levels are able to "rescue" sprint performance after heat exposure, at least not within the time frame measured (1 h after heat treatment). It is possible that individuals with high Hsp70 regain sprint performance with longer recovery periods.

In summary, we have used the widespread lizard *S. occidentalis* to demonstrate geographic variation in sprint performance and muscle Hsp70 levels after heat exposure. Some of this variation is explained by differences in collection site elevation, while some is related to underlying regional differences among lizards. Pooling lizards from all collection sites, there is a negative relationship between sprint speed and hind-limb muscle Hsp70 expression after heat exposure, suggesting that an increase in Hsp70 did not compensate for the adverse effects of high-temperature exposure, at least within the time frame assessed. Further investigation is needed to elucidate the possible mechanisms underlying this correlation as well as the environmental and genetic contributions to geographic variation in sprint speed and Hsp70 expression. Ultimately, if high-tem-

perature exposure decreases the ability of these organisms to elude predators or capture prey, this could negatively affect individual fitness and population viability in the face of further increases in air temperatures due to global climate change.

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