

# Lizards fail to plastically adjust nesting behavior or thermal tolerance as needed to buffer populations from climate warming

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## Abstract

Although observations suggest the potential for phenotypic plasticity to allow adaptive responses to climate change, few experiments have assessed that potential. Modeling suggests that *Sceloporus tristichus* lizards will need increased nest depth, shade cover, or embryonic thermal tolerance to avoid reproductive failure resulting from climate change. To test for such plasticity, we experimentally examined how maternal temperatures affect nesting behavior and embryonic thermal sensitivity. The temperature regime that females experienced while gravid did not affect nesting behavior, but warmer temperatures at the time of nesting reduced nest depth. Additionally, embryos from heat-stressed mothers displayed increased sensitivity to high-temperature exposure. Simulations suggest that critically low temperatures, rather than high temperatures, historically limit development of our study population. Thus, the plasticity needed to buffer this population has not been under selection. Plasticity will likely fail to compensate for ongoing climate change when such change results in novel stressors.

**Keywords:** incubation, mechanistic model, phenotypic plasticity, *Sceloporus tristichus*, *Sceloporus undulatus*

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## Introduction

Many organisms respond to climate change through phenotypic plasticity including phenological shifts, morphological changes, physiological acclimation, and shifts in behavior (Parmesan, 2006; Merilä & Hendry, 2014; Urban *et al.*, 2014; Seebacher *et al.*, 2015). Behavioral plasticity may be an especially powerful mechanism for buffering environmental variation because behaviors can shift rapidly and reversibly (Charmantier *et al.*, 2008; Telemeco *et al.*, 2009; Huey *et al.*, 2012; Zuk *et al.*, 2014; Muñoz *et al.*, 2015). In general, such phenotypic plasticity is assumed to promote population persistence in the face of climate change. However, the adaptive value of plasticity in response to ongoing climate change has rarely been directly tested (Merilä & Hendry, 2014; Duputié *et al.*, 2015).

For current plasticity to buffer populations from climate change, the necessary phenotypic response must have been historically selected or exist by chance (i.e., exaptation). This constraint should be general and apply across environmental characteristics and phenotypes

(Levins, 1968), and the chance existence of adaptive plasticity to novel environments should be rare. Thus, adaptive plasticity should generally exist only when future environments lie within the bounds of variation experienced in the past. Moreover, the relationship between the environmental cue and phenotypic response must persist into the future for plasticity to remain adaptive (Chevin *et al.*, 2010). For example, because recent environments are within the historical range of variation, great tits (*Parus major*) in the United Kingdom have successfully compensated for climate change by plastically adjusting their reproductive timing to coincide with food availability (Charmantier *et al.*, 2008). However, further directional climate change could exceed the capacity for plasticity and cause population collapse (Charmantier *et al.*, 2008; Chevin *et al.*, 2010). Plasticity will be least likely to buffer populations when climate change results in novel environments. Given these limitations, we hypothesize that phenotypic plasticity compensating for climate change might be the exception rather than the rule (Chevin *et al.*, 2010; Valladares *et al.*, 2014).

Models that estimate fitness based on phenotypes and environmental conditions can predict the direction and magnitude of phenotypic plasticity needed to buffer populations from climate change (e.g., Kearney & Porter, 2009; Kearney, 2013; Duputié *et al.*, 2015; Levy

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*et al.*, 2015). For example, a model for lizards of the *Sceloporus undulatus* species complex predicts reproductive failure across 35% of the geographic range by 2100, resulting from fatal heating of embryos in soil (Levy *et al.*, 2015). However, if females nest in 25% shadier microhabitats or 3 cm deeper, or if the thermal tolerance of embryos increases, predictions reverse and populations are predicted to benefit from climate change (Levy *et al.*, 2015). Thus, the predicted fate of these populations rests on whether embryos can tolerate future environments through either acclimation or mothers laying eggs in cooler places.

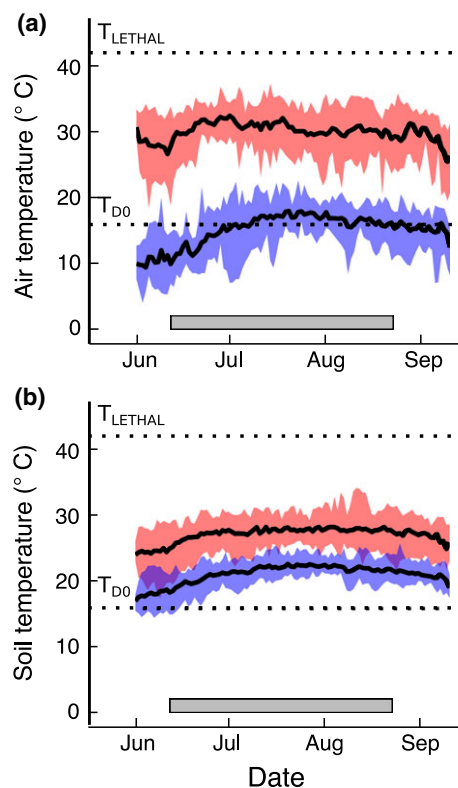
We tested for such plasticity in a population of *S. tristichus*, the westernmost evolutionary species (Coyne & Orr, 2004) of the *S. undulatus* complex (Leaché, 2009). Compared to other *S. undulatus* lizards, *S. tristichus* display similar preferred temperatures and thermal limits throughout ontogeny, as well as similar ecology (Angilletta *et al.*, 2013; Buckley *et al.*, 2015). Moreover, our study population is among those predicted to experience fatal temperatures by 2100 (Levy *et al.*, 2015). We first tested the hypothesis that females would construct deeper or shadier nests to compensate for warmer conditions by examining the effect of temperature during pregnancy (optimal and heat-stress treatments) and immediately prior to oviposition on nest depth and shade cover selection. Next, to test the hypothesis that acclimation of embryonic thermal tolerance can rescue embryos from elevated nest temperature, we collected eggs from these females and quantified their tolerance to rare extreme-heat events after heat acclimation. Finally, we used weather records and simulation models to estimate developmental success and energetics across thermal environments. Using this information, we examined the selective pressures that might have resulted in the behaviors and thermal tolerances that we observed. Together, these data fail to support the hypothesis that current plasticity in nesting behavior or thermal tolerance will promote persistence of this population during future warming.

## Materials and methods

### Experimental study of nesting behavior

We collected gravid female *S. tristichus* ( $N = 50$ ) in May of 2015 from Navajo Co, AZ (34.2021,  $-110.0779$ ,  $\approx 1900$  m elev.) and transported them to Arizona State University (ASU). We assessed reproductive status at the time of collection by palpating the abdomen (Sloan & Baird, 1999). Lizards were maintained according to established protocols (Angilletta *et al.*, 2013; Levy *et al.*, 2015). While gravid, lizards were exposed to one of two thermal treatments created in environmental chambers (Percival DR-36VL; Percival Scientific, Perry, IA, USA).

The first thermal treatment modeled an environment in which lizards can thermoregulate during activity. We maintained lizards at a preferred temperature of 33 °C (Buckley *et al.*, 2015) for 8 h day<sup>-1</sup> and at 20 °C for the remainder of the diel cycle (Fig. S1a). We chose this cycle because eight h of thermoregulation maximizes the daily rate of energy acquisition (J. D. Borchert, O. Levy, T. Rusch, L. Buckley, and M. J. Angilletta, unpublished data), and 20 °C approximates temperatures at night at the site of collection (see Historical climate and Fig. 1). The second thermal treatment modeled what lizards might experience after climate warming. Because lizards behaviorally thermoregulate, a uniform temperature increase will not result in a uniform body temperature increase. Rather, lizards will maintain their preferred temperature as long as possible, after which body temperature may increase (Adolph & Porter, 1993; Buckley *et al.*, 2015). Thus, lizards were maintained at 33 °C for 8 h day<sup>-1</sup>, but this was divided into two periods of 4 h separated by a period of 3 h when temperatures rose slowly to 39 °C before returning to



**Fig. 1** Air temperatures (at 1.5 m above the soil surface) during incubation never approach critical high temperatures, but can frequently become stressfully cold (a). Soil temperatures (10 cm below bare soil) indicate that deep nests will be protected from such cold temperatures (b). We depict the means (black lines) and ranges (shaded) of daily maximum and minimum temperatures over our 12-year dataset (2004–2015) from a weather station in Payson, AZ. The dotted, horizontal lines are the lethal limit for development ( $T_{LETHAL}$ ) and the developmental zero temperature ( $T_{D0}$ ) for *Sceloporus tristichus*. Horizontal bars represent the average incubation period (12 June–23 August).

33 °C, resulting in 11 warm hours (Fig. S1b). This treatment includes temperatures that exceed the preferred range but do not exceed the critical thermal maximum (Buckley *et al.*, 2015). When lizards cannot maintain body temperatures within their preferred range, they frequently cease activity (Adolph & Porter, 1993; Sinervo *et al.*, 2010). Therefore, our second treatment models a relatively extreme, but realistic, scenario where lizards either reduce their thermoregulatory precision during activity because preferred microclimates become rare (Sears & Angilletta, 2015) or shelters become warmer than the preferred temperature. For both treatments, the light cycle was 12L : 12D and the relative humidity was 50%, which approximate conditions during spring where the lizards were collected.

Twice per week, we assessed the developmental state of eggs by palpating each female's abdomen (Sloan & Baird, 1999). One week after first detecting oblong and turgid eggs (i.e., ready for oviposition, Sloan & Baird, 1999), we moved each female to its own outdoor arena (150 × 60 × 60 cm) in Tempe, AZ, that provided 30-cm-deep soil and a gradient of shade on the walls and ceiling: 30%, 60%, and 90% (see Appendix S1 for details). Even though the shade treatments provided a range of thermal microenvironments, temperatures within the arenas were generally hotter than those experienced by lizards from our high-elevation population and thus simulated nesting conditions during particularly warm years (compare Figs 1 and S2). While in the arenas, lizards were inspected three times per day for evidence of nesting: 0530–0600 h, 1000–1100 h, and 1800–1900 h. On many days, lizards were also inspected between 2200 and 0200 h. As soon as nesting was detected, we removed females and carefully sifted the soil to locate their eggs. We carefully collected the eggs and recorded the level of shade over the nest (i.e., 30%, 60%, or 90%). We quantified nest depth as the distance from the soil surface to the bottom of the nest cavity and nest position along the shade gradient as the distance from the center of the nest to the far edge of the arena, where 90% shade was available, along its long axis. Both the mother and her eggs were returned to the laboratory on the same day.

For three randomly selected arenas, we recorded thermal profiles at 15-min intervals using thermistor probes attached to factory-calibrated data loggers (PT907 probes attached to XR5-SE data loggers, Pace Scientific Inc., Mooresville, NC, USA). Probes were positioned in the center of each shade treatment, recording soil temperatures at the surface and 5 cm below. In one arena, we also recorded air temperature ≈15 cm above the soil surface under 90% and 30% shade.

We performed statistical analyses in the R programming environment (version 3.1.3, R Core Team, 2015). For these analyses, we included only females that exhibited normal nesting behavior (i.e., placed their eggs in a cluster beneath the soil surface). We constructed general linear models using the 'lm' function in R to test for effects of thermal treatment during egg development on the depth and position of the nest, with time in treatment and time in arena included as covariates. We next used contingency tables to test the hypothesis that females from each treatment nested randomly with respect to shade cover (functions 'table' and 'chisq.test').

Finally, we tested the hypothesis that temperatures experienced shortly before oviposition cued variation in nesting behavior using linear models including mean temperature on the day before nesting, maximum temperature on the day before nesting, and minimum temperature on the day of nesting under 30% shade (all females were in their nesting arena >1 d prior to ovipositing). Because females constructed nests in the morning, minimum temperature approximates temperature at nesting. We focused on temperatures under 30% shade because these were the most extreme temperatures available to females and should indicate the potential for heat stress during development. For all linear models, we used AICc to select the preferred reduced models ('aictab' in the AICcmodavg library; Mazerolle, 2015) and we assessed significance using type-3 sum of squares ('ANOVA' function in the car library; Fox & Weisberg, 2011).

### *Thermal sensitivity of embryonic survival*

On the same day that eggs were collected, they were placed in an incubation regime that mimicked average nest conditions predicted for the period between 2080 and 2100 at our study site (diel cycle from 18.5 to 38.1 °C, mean = 22.6 °C, Fig. S3; Levy *et al.*, 2015). Eggs remained in this regime for approximately 75% of development before being exposed to an acute heat stress (see below). The initial incubation regime was designed to assess whether or not early incubation conditions alter embryonic thermal tolerance from that previously estimated (Angilletta *et al.*, 2013; Levy *et al.*, 2015). The elevated temperatures were not stressfully hot and may improve development and survival (Levy *et al.*, 2015). All other conditions followed standard protocols (Angilletta *et al.*, 2013; Levy *et al.*, 2015).

On July 24, eggs ( $N = 28$ ) were moved to one of three heat-stress treatments. These treatments (Fig. S3) had the same minimal temperature as the initial treatment, but peaked at 40, 41.5, or 43 °C ( $N = 8, 10,$  and  $10$  eggs, respectively) instead of 38 °C. At the onset of this experiment, eggs had incubated 41 d, on average (SD = 7.75 d, range = 13–54 d), which translates to ~75% of development (estimated using relationship in Fig. S4). Prior to the experiment, we confirmed that all eggs were alive by measuring cardiac activity with a commercially available system of infrared sensors (Buddy Egg Monitor, Avitronics, Cornwall, UK, see Angilletta *et al.*, 2013; for details). We maintained eggs in the heat-stress treatments for 7 d. During the cool period of each day, we re-measured cardiac activity to see whether embryos were still alive. We analyzed the data using a quasibinomial GLM ('glm' function in R, quasibinomial to account for overdispersion) with proportion of embryos alive each day as the dependent variable and day of the experiment included as a covariate. Because AIC is undefined for quasibinomial models, we instead used backwards selection based on  $P$  values to select the preferred reduced model (Zuur *et al.*, 2009).

### *Body temperature while nesting*

To ascertain whether or not females became thermally stressed while digging their nests, which might affect nesting

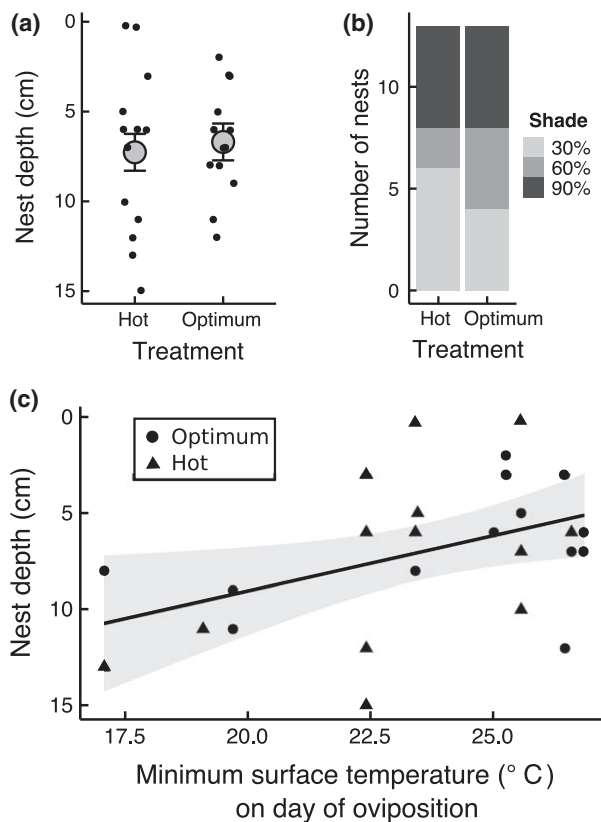
behavior, we estimated operative temperatures of nesting lizards using a biophysical model (Fei *et al.*, 2012) modified by Levy *et al.* (2015) to fit *Sceloporus* lizards. We used temperatures within the nesting arenas for most environmental variables (e.g., soil surface, soil, and near-surface air temperature), but acquired air temperature (1.5 m) and solar radiation data from a nearby weather station (Mesa, AZ,  $\approx 5$  km from our nesting arena site) managed by the Arizona Meteorological Network (AZMET, <http://cals.arizona.edu/azmet>). Parameters for the model are described in Table S1. For each female, we calculated operative temperatures every minute between the time that we last noted that she was gravid and the time that we discovered that she had oviposited (periods  $\leq 6$  h). Calculations at a resolution of minutes are needed to account for thermal inertia of the organism even though environmental data were measured more coarsely. For these calculations, we assumed that females were digging their nest and thus exposed to the nest's shade environment. Calculating body temperature across these time periods enabled us to conservatively assess the potential for heat stress during nesting.

### Historical climate

We used historical climate to infer recent selective pressures on nesting behavior and thermal tolerance in our focal population. We downloaded hourly records of air temperature (1.5 m above soil surface) and soil temperature (10 cm) for 2004–2015 in Payson, AZ (34.2325,  $-111.3442$ , 1478 m, AZMET network; temperature for additional heights, depths, and years was unavailable). This station was the closest to our focal population ( $\approx 200$  km), is at a similar latitude and elevation, and has similar habitat, and *S. tristichus* can be found in the region. We used air temperature as a proxy for the thermal environment experienced by adult females and eggs in shallow nests. In a similar habitat, adult female *S. undulatus* perch at an average height of 75 cm above the ground (Pounds & Jackson, 1983). The soil temperature at a depth of 10 cm was used as a proxy for conditions experienced by eggs in deep nests (Fig. 2a). We assessed the risk of critically high or low temperatures occurring during embryonic development, assuming development occurs during the average developmental period observed at our site (12 June–23 August). We also examined whether thermal conditions experienced by mothers before oviposition predict thermal conditions experienced by embryos in the nest by testing for correlations between the thermal environment (means and extremes) experienced by females over various windows of time prior to oviposition and that experienced by embryos during development. See Appendix S1 for details.

### Development simulations

To better understand the selective pressures on nesting behavior, we used computer simulations to explore the effects of the grand mean temperature and mean diel range of temperatures on incubation period, energy consumption, and embryonic mortality. Grand mean nest temperature is expected to vary spatially as a result of nest shade cover and moisture, and



**Fig. 2** Temperature treatment while females were gravid (hot vs. optimum) did not affect (a) nest depth (black points: jittered data for each nest; large gray points: least-squares means  $\pm$  SE) or (b) shade cover over nests, but (c) females dug shallower nests in response to warmer temperatures when nesting (line of best fit and 95% confidence interval indicated).

temporally as a result of climate change. Diel thermal range is a useful proxy for nest depth because it reduces with soil depth, while daily mean temperature is generally unaffected (Campbell & Norman, 1998; and Figs 1 and S2). We used published data for *S. undulatus* complex lizards to estimate the thermal sensitivity of developmental rate, metabolic rate, and survivorship (Andrews *et al.*, 2000; Angilletta *et al.*, 2006, 2000; Levy *et al.*, 2015; Oufiero & Angilletta, 2006; Parker & Andrews, 2007; Sexton & Marion, 1974; Warner & Andrews, 2003, see Appendix S1).

We simulated hourly temperatures ( $T$ ) throughout development using the following equation (Campbell & Norman, 1998):

$$T = T_{\text{AVE}} + A(0) \sin(\omega(t - t_0)),$$

where  $T_{\text{AVE}}$  is mean temperature,  $A(0)$  is the diel amplitude ( $1/2$  diel thermal range),  $\omega$  is the angular frequency,  $t$  is time of day, and  $t_0$  is a constant that syncs the sin wave with time of day. For our simulations, we set  $\omega = 2 \times 10^{-7} \text{ s}^{-1}$  and  $t_0 = 8$  (Campbell & Norman, 1998). The diel pattern of temperature remained fixed throughout development, which enabled better comparison to experimental data. We simulated temperatures throughout development for every combination of

$T_{AVE}$  from 10 to 30 °C and  $A(0)$  from 0 to 15 °C, which encompasses the average thermal variation during the reproductive season in Payson, AZ (Fig. 1) and that predicted for 2100 in northern Arizona (Levy *et al.*, 2015). We assumed zero developmental success for any combinations of  $T_{AVE}$  and  $A(0)$  that exceeded  $T_{LETHAL}$  (42 °C) or never rose above the minimum temperature for development ( $T_{D0} = 15.9$  °C). For each hour, we used temperature to estimate % development completed and energy consumed. We continued until development reached 100%, at which point we summed the time and energy needed to complete development. Finally, we estimated probability of mortality, given the minimal and maximal temperatures during development. Simulations were performed in R using the 'foreach' function from the *foreach* library (Revolution Analytics & Weston, 2014).

## Results

### Nesting behavior

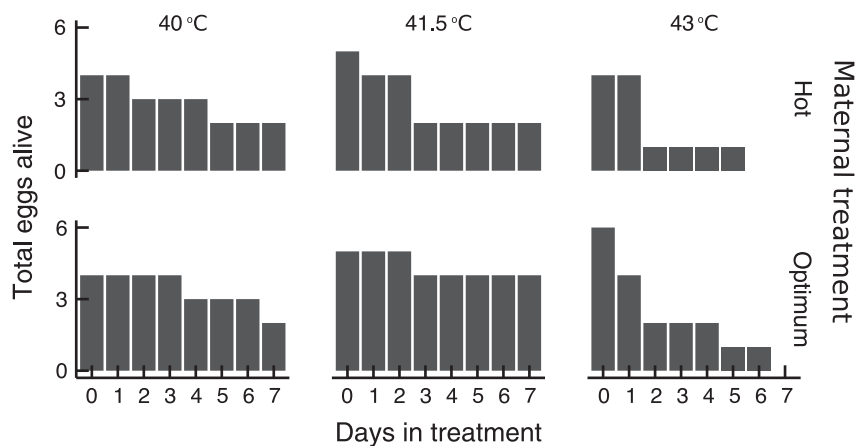
Thirty females oviposited within the arenas, of which 26 displayed normal behavior (i.e., placed their eggs in a cluster and covered with soil, rather than scattering eggs on the surface). Maternal treatment in the laboratory did not influence the vertical depth ( $F_{1,24} = 0.14$ ,  $P = 0.71$ , Fig. 2a) or position along the shade gradient ( $F_{1,23} = 0.02$ ,  $P = 0.90$ ) chosen when nesting. Moreover, females from both treatments nested randomly with respect to shade (hot:  $\chi^2 = 2$ ,  $df = 2$ ,  $P = 0.37$ , optimum:  $\chi^2 = 0.15$ ,  $df = 2$ ,  $P = 0.93$ , Fig. 2b). On average, females were exposed to their thermal treatment for 19.3 d (1.0 SD = 4.4 d), and were in their nesting arena for 7.7 d (1.0 SD = 4.4 d). Even so, neither days in treatment ( $F_{1,22} = 0.06$ ,  $P = 0.80$ ) nor days in nesting arena ( $F_{1,22} = 3.94$ ,  $P = 0.06$ ) affected the depth of the nest. Similarly, neither covariate affected the shading of the nest (days in treatment:  $F_{1,21} = 0.44$ ,  $P = 0.51$ ; days in

arena:  $F_{1,21} = 1.49$ ,  $P = 0.24$ ). Moreover, inclusion of these covariates did not improve model performance ( $\Delta AICc < 2$ ).

Temperature during oviposition influenced nesting behavior, but not in the direction predicted to buffer embryos from climate warming. The most likely model of nest depth included only the minimal air temperature on the day of nesting, and lizards constructed deeper nests when mornings were colder ( $F_{1,24} = 5.76$ ,  $P = 0.02$ , Fig. 2c). For nest shade, the most likely model included effects of mean and maximal temperatures, but neither effect was statistically significant (mean temperature:  $F_{1,22} = 1.70$ ,  $P = 0.21$ ; maximal temperature:  $F_{1,22} = 1.39$ ,  $P = 0.25$ ). Neither maternal treatment nor its interaction with temperature proximate to oviposition was included in the preferred models.

### Embryonic thermal sensitivity

The survival of embryos depended on interactions between the thermal treatment during incubation and the thermal treatment to which mothers were exposed ( $\chi^2 = 4.14$ ,  $df = 2$ ,  $P < 0.01$ ), and between incubation treatment and the number of days embryos were exposed to high temperatures ( $\chi^2 = 2.90$ ,  $df = 2$ ,  $P = 0.03$ ). We further explored these interactions using pairwise tests. Hotter conditions during incubation reduced the survival of embryos from mothers exposed to both the hot and optimal treatments (optimal:  $\chi^2 = 117.81$ ,  $df = 2$ ,  $P < 0.01$ ; heat stress:  $\chi^2 = 19.93$ ,  $df = 2$ ,  $P < 0.01$ , Fig. 3), and survival of all embryos fell with repeated exposure to high temperatures (40 °C:  $\chi^2 = 31.65$ ,  $df = 1$ ,  $P < 0.01$ ; 41.5 °C:  $\chi^2 = 13.68$ ,  $df = 1$ ,  $P < 0.01$ , 43 °C:  $\chi^2 = 27.93$ ,  $df = 1$ ,  $P < 0.01$ ; Fig. 3). Compared to embryos exposed to optimal



**Fig. 3** *Sceloporus tristichus* embryos become increasingly sensitive to high temperature [daily maxima of 40 °C (left), 41.5 °C (center), or 43 °C (right)] with increasing days of exposure, and this effect is exacerbated when mothers are exposed to stressful temperatures while gravid. Data are the number of eggs alive after each day of exposure. See Fig. S5 for a plot combining maternal treatments.

temperatures *in utero*, those exposed to high temperatures *in utero* survived poorly when exposed to 40 °C ( $\chi^2 = 6.72$ ,  $df = 1$ ,  $P < 0.01$ ) and 41 °C ( $\chi^2 = 32.64$ ,  $df = 1$ ,  $P < 0.01$ ) later in development, but not when exposed to 43 °C ( $\chi^2 = 0$ ,  $df = 1$ ,  $P = 1$ , Fig. 3). In general, the effects of high temperatures on survival were similar to those reported previously (Angilletta *et al.*, 2013; Levy *et al.*, 2015). Thus, embryos exhibited no heat hardening in response to elevated temperatures experienced in the uterus or during the first 50–75% of incubation.

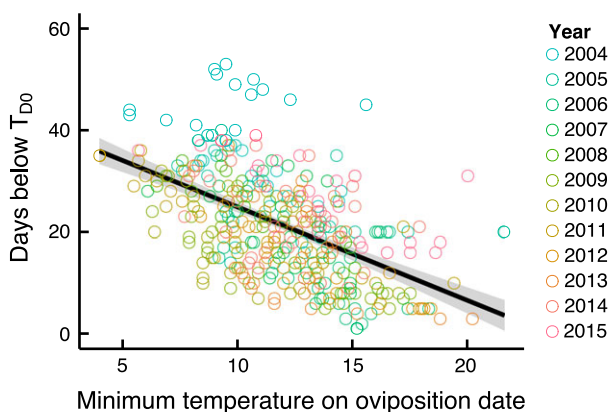
#### Body temperature while nesting

Operative temperatures estimated for nesting females did not approach critical thermal limits (Fig. S6). The minimal and maximal operative temperature averaged 24.5 °C (SD = 2.3 °C, range = 20.2–28.8 °C) and 28.1 °C (SD = 2.5 °C, range = 24.1–32.5 °C), respectively. These temperatures lie well within the critical limits of 10.7 and 41.5 °C, and just below the mean temperature selected in a thermal gradient during early morning, 29.8 °C (Buckley *et al.*, 2015).

#### Historical climate

During the period when embryos develop in the nest, neither 1.5-m air nor 10-cm soil temperature in the field approached critical highs for development over our 12-year dataset (Figs 1 and S7). The highest temperatures of air and soil during the incubation period were 37.3 and 34.2 °C, respectively; both are substantially below the lethal temperature of embryos, 42 °C (Levy *et al.*, 2015). By contrast, stressfully low temperatures were frequent (Figs 1 and S7), with minimal air and soil temperatures across the incubation period being 6.4 and 15.0 °C, respectively. Every year, air temperature fell below the minimal temperature for development (15.9 °C) multiple times during the average incubation period. Soil temperatures, however, were nearly completely buffered from this low (Figs 1 and S7).

Only the minimal air temperature on the day of oviposition provided a cue of future conditions; if a mother nested on a colder morning, her embryos were more likely to experience temperatures that fell below the minimum for development (15.9 °C) if placed within shallow nests (entirety of incubation:  $\chi^2 = 65.30$ ,  $df = 1$ ,  $P < 0.01$ , Fig. S8; the first portion of incubation:  $\chi^2 = 30.52$ ,  $df = 1$ ,  $P < 0.01$ , Fig. 4). Otherwise, temperatures experienced by females poorly predicted temperatures experienced by embryos. Average thermal conditions experienced by females and eggs were never correlated ( $P > 0.2$  for all, Fig. S9 displays correlations



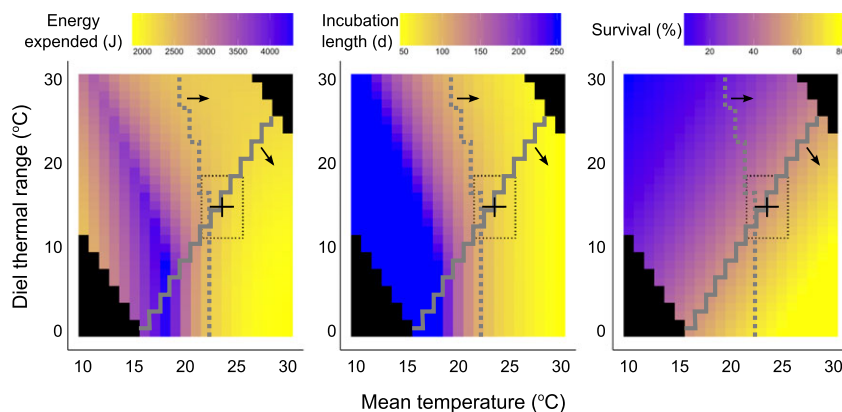
**Fig. 4** Minimal temperature at oviposition predicts the number of days that *Sceloporus tristichus* eggs could experience stressfully cold temperatures if placed in shallow nests (line of best fit and 95% confidence interval indicated). We assume that incubation could initiate each day of June across our 12-year dataset (points). We depict the number of days from the onset of incubation until the end of July with air temperature (at 1.5 m in Payson, AZ) below the developmental zero temperature (15.9 °C). See Fig. S8 for a plot spanning the entire incubation period.

between the thermal environment of females and that of embryos). Although female and egg absolute maximal and minimal air temperatures were positively correlated ( $P < 0.01$ , Fig. S9), correlations were generally weak ( $< 0.25$ ).

#### Development simulations

Both developmental rate (dec. %  $h^{-1}$ ) and energy expenditure ( $J$ ) increased with incubation temperature (Figs S4 and S10 display these relationships). As a result, the incubation period declined as grand mean nest temperature increased, and shallow nests shortened incubation by exposing embryos to higher temperatures each day (Fig. 5). Total energy consumed during development generally declined with increasing temperature (Fig. 5, left) because incubation shortened (Fig. 5, middle). Even so, daily exposure to low temperatures in shallow nests can slow metabolism sufficiently to outweigh the energetic cost of prolonged development, particularly when mean temperatures are  $< 20$  °C (Fig. 5, left and middle). Embryos are most likely to survive in warm, deep nests, which are not predicted to reach lethal temperatures (Fig. 5, right).

All things considered, warm, deep nests optimize development because they minimize incubation period and energetic cost while maximizing the probability of survival (Fig. 5). Although shallow nests can reduce energetic cost and incubation period in cool



**Fig. 5** Simulation models predict that avoiding the survival cost of stressfully cold temperatures would result in a negative correlation between nest depth and temperature at nesting, as observed. Plots depict how mean temperature and diel thermal range (proxy for nest depth, larger thermal range = shallower nest) influence the total energetic cost of development (left), duration of development (center), and survival to hatching (right) in *Sceloporus undulatus* species-group lizards. Lighter colors (more yellow) denote more favorable conditions. We indicate the mean air temperature (1.5 m) and mean diel thermal range (+ symbol)  $\pm$  one SD (dotted box) during the average incubation period (12 June–23 August) at Payson, AZ. Black areas mask regions of state space where development is not possible [top right exceeds  $T_{LETHAL}$  (42 °C) and the bottom left never exceeds  $T_{D0}$  (15.9 °C)]. The gray lines denote the boundaries for conditions desirable for development, with arrows pointing to the desirable side (dotted line: development faster than 100 d; solid line: egg temperatures remain above  $T_{D0}$ ).

environments, such nests are predicted to experience low temperatures that increase mortality (Fig. 5) and incubation may be prohibitively long (>100 days, Andrews *et al.*, 2000; Angilletta *et al.*, 2000; Oufiero & Angilletta, 2006; Parker & Andrews, 2007; Sexton & Marion, 1974; Warner & Andrews, 2003). As nest temperatures warm, nest depth has little survival benefit until it eliminates heat stress (Fig. 5). In general, historical temperatures during development fall in a region of state space where cold stress, but not heat stress, is a frequent challenge for embryos in shallow nests (Fig. 5).

## Discussion

Both mothers and embryos of *S. tristichus* displayed responses to elevated temperatures that should increase the population's susceptibility to climate change, rather than reduce it. Females dug shallower nests in response to warmer conditions during oviposition which increases the risk that offspring will experience lethally high temperatures predicted to result from climate change by 2100 (i.e., Levy *et al.*, 2015), even though such temperatures are not a current threat. By contrast, females did not alter their nesting behavior in response to temperatures maintained in the laboratory during the majority of gravidity. Biophysical modeling indicated that nesting females were near their preferred temperature and more than 10 °C below their critical thermal

maximum; thus, females did not construct shallow nests to avoid overheating while digging. Moreover, offspring of heat-stressed mothers were more sensitive to high temperatures during incubation. Embryos may have been damaged *in utero* by either the direct effects of high maternal body temperatures or as an indirect result of reduced gas or hydric exchange between mother and embryo.

To understand the adaptive value of phenotypic plasticity, one needs to understand the selective environment in which such plasticity evolved (e.g., Falconer & Mackay, 1996; West-Eberhard, 2003; Piersma & Van Gils, 2011). During the 12 years for which we have weather data, neither air nor soil temperature approached the high, lethal limit of embryos during development. Thus, this population of lizards has not experienced selection to avoid hot nests in recent history. Although shallow nests could increase offspring fitness by exposing them to beneficially warm temperatures for part of the day (Andrews *et al.*, 2000; Parker & Andrews, 2007; Levy *et al.*, 2015), they also increase the risk of eggs experiencing temperatures below the minimum for development. A single exposure to the developmental minimum can reduce survival to  $\approx 50\%$  (Levy *et al.*, 2015), and those lizards that hatch may be of poor quality (Qualls & Andrews, 1999; Parker & Andrews, 2007; Telemeco *et al.*, 2010). Thus, by digging deeper nests, females could buffer their offspring from the negative fitness consequences of low temperatures.

For adaptive plasticity to evolve, the current environment must reliably predict the future environment (Levins, 1968; Moran, 1992; Kingsolver & Huey, 1998; West-Eberhard, 2003; Ezard *et al.*, 2014). Our results suggest that average thermal conditions before nesting poorly predict the thermal environment experienced by embryos, even if females integrate thermal data over long periods. This is because development is sufficiently long in reptiles (2–3 months) that temperatures prior to oviposition are not autocorrelated with those late in development (Kingsolver & Huey, 1998; Telemeco *et al.*, 2013; Dowd *et al.*, 2015). However, temperatures just prior to oviposition are indicative of those early in development (Dowd *et al.*, 2015): The minimal temperature at nesting reliably predicted the incidence of stressfully low temperatures, which primarily occur early in development. Thus, recent conditions have been conducive for the evolution of nesting plasticity that reduces the chance of embryos experiencing cold stress. However, adaptive plasticity of nesting behavior to reduce heat stress likely could not evolve, even if embryos experienced stressfully high temperatures. This constraint exists because temperatures experienced by mothers do not correlate with temperatures experienced by embryos late in development, when stressful high temperatures are most likely to occur.

Shallow nests, which have high thermal variance, reduce the probability of survival when mean temperatures are either low or high. This observation raises a question: Why should females ever place their eggs in a shallow nest? The most parsimonious answer likely involves trade-offs between the cost of digging and the benefit of having offspring develop in a deep nest (Roff, 1992; Stearns, 1992). Digging a nest cavity likely imposes costs such as loss of energy, missed opportunities for foraging or thermoregulation, or greater exposure to predators (Spencer, 2002; Angilletta *et al.*, 2009). Our simulations demonstrate that, within the range of temperatures historically experienced by our focal population, a deep nest provides little benefit when average temperatures are relatively warm. Rather, deep nests become beneficial only when average temperatures are low. This trade-off should select for females that dig deep nests only when exposed to low temperatures, as we observed.

Although we observed no evidence of plasticity suitable to compensate for predicted climate warming, we did observe large variation in nest depth and shade cover that could enable an evolutionary response if some proportion is heritable (Lynch & Lande, 1993; Chevin *et al.*, 2010; Zuk *et al.*, 2014). Unfortunately, the heritability of nesting behavior in reptiles is poorly characterized. One exception, however, is a population

of painted turtles (*Chrysemys picta*) for which heritabilities of nesting date and shade cover ranged from 0 for both traits during years with cold winters to 0.166 and 0.188, respectively, for warm winter years (McGaugh *et al.*, 2010). If the variation in nesting behavior of *S. tristichus* is equally heritable, the population could evolve to dig nests that are 3 cm deeper or 25% more shaded in six generations, which translates to approximately 18 years (assuming constant heritability of 0.188, constant selection gradients of 3 and 0.25, and a three-year generation time) (Tinkle & Ballinger, 1972; Falconer & Mackay, 1996). Similarly, behavior and morphology in *S. undulatus* complex lizards have evolved over the last 70 years in response to the introduction of a novel predator (Langkilde, 2009). Thus, rapid evolution of nesting behavior in response to climate change seems plausible.

Although inappropriate to buffer populations from predicted climate change, the nesting plasticity that we observed might enable females to buffer their offspring from critically low temperatures, which have been a frequent challenge. Over the course of the coming century, the adaptive value of behavioral plasticity in this population may reverse directions; digging shallow nests in warm years appears adaptive when high temperatures are below the fatal threshold (present study) but would be maladaptive if nests experience fatally high temperatures, as predicted for the future (Levy *et al.*, 2015). This result highlights the importance of considering plasticity in the context within which it evolved. For plasticity to help a population persist during environmental change, future environmental conditions should lie within the range of conditions that originally selected for the current reaction norm (Charmantier *et al.*, 2008; Chevin *et al.*, 2010; Merilä & Hendry, 2014). Phenotypic plasticity is thus unlikely to alleviate the impacts of climate change when patterns of stress in future climates have no analogue to those experienced in the past, such as novel instances of critically high temperatures during reproduction and development.

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## Supporting Information

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

**Appendix S1.** Nesting arenas, historic climate analyses and development simulations.

**Table S1.** Parameters used to calculate body temperature of *S. tristichus* ( $T_b$ ) during nesting.

**Figure S1.** Schematics of maternal thermal treatments (A and B), nesting arenas (C), and the layout of arenas on the landscape (D, 10 blocks of 4 arenas).

**Figure S2.** Temperatures for the entire nesting season within the experimental nesting arenas under each shade type, and from a nearby weather station (Mesa, AZ) at the soil surface (touching the surface in the arenas and 1.5 m air temperature for Mesa) or below the soil surface (5 cm below for the arenas and 10 cm below for Mesa).

**Figure S3.** Egg incubation treatments. For the majority of incubation, all eggs were exposed to the treatment depicted by the solid black line (peaks at 38 °C), which models the average conditions that *Sceloporus tristichus* eggs from our study population are predicted to experience between 2080 and 2100. The shades of red depict thermal stress treatments to which eggs were exposed for 7 d.

**Figure S4.** Plots showing the effect of temperature on development in *Sceloporus undulatus* species-group lizards.

**Figure S5.** The magnitude and duration of high-temperature exposure interact to affect *Sceloporus tristichus* embryo survival.

**Figure S6.** Operant temperature traces for each female during her inferred nesting window.

**Figure S7.** Neither soil temperature (10 cm) nor air temperature (1.5 m) during the incubation period ever approached critical high temperatures ( $T_{LETHAL}$ ) for *Sceloporus tristichus* development.

**Figure S8.** Minimum temperature at oviposition is a good predictor of the number of days that *Sceloporus tristichus* eggs could experience stressfully cold temperatures if placed within shallow nests. Data are for the entire reproductive season.

**Figure S9.** Average air temperature prior to oviposition poorly predicts average thermal conditions within the soil during the incubation period at Payson, AZ, regardless of the size of the pre-oviposition window considered ( $P > 0.35$  for all).

**Figure S10.** Effect of temperature on the metabolic rate of *Sceloporus undulatus* species-group eggs.