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Thermal physiology and thermoregulatory behaviour exhibit low heritability despite genetic divergence between lizard populations

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Ectothermic species are particularly sensitive to changes in temperature and may adapt to changes in thermal environments through evolutionary shifts in thermal physiology or thermoregulatory behaviour. Nevertheless, the heritability of thermal traits, which sets a limit on evolutionary potential, remains largely unexplored. In this study, we captured brown anole lizards (*Anolis sagrei*) from two populations that occur in contrasting thermal environments. We raised offspring from these populations in a laboratory common garden and compared the shape of their thermal performance curves to test for genetic divergence in thermal physiology. Thermal performance curves differed between populations in a common garden in ways partially consistent with divergent patterns of natural selection experienced by the source populations, implying that they had evolved in response to selection. Next, we estimated the heritability of thermal performance curves and of several traits related to thermoregulatory behaviour. We did not detect significant heritability in most components of the thermal performance curve or in several aspects of thermoregulatory behaviour, suggesting that contemporary selection is unlikely to result in rapid evolution. Our results indicate that the response to selection may be slow in the brown anole and that evolutionary change is unlikely to keep pace with current rates of environmental change.

1. Introduction

Anthropogenic climate change is resulting in rapid shifts in the thermal environments experienced by terrestrial ectotherms, many of which have limited ability to disperse and may need to rely on *in situ* adaptation to avoid extinction [1–4]. When local thermal environments become suboptimal, evolutionary adaptation in both thermal physiology and thermoregulatory behaviour may help ameliorate the negative impacts of environmental change.

Evolutionary shifts in thermal physiology manifest at the organismal level as changes in the shape of thermal performance curves. Thermal performance curves describe the relationship between body temperature and performance of an ecologically relevant task such as locomotion, digestive efficiency or foraging rate [5–7]. To the extent that the internal body temperatures of individuals covary with environmental temperatures, increases or decreases in the mean and variance of environmental temperature should lead to modifications of thermal performance curves [8]. For example, a change in the variance of body temperatures experienced by individuals should select for a

shift in curve breadth, whereas a change in mean body temperature should select for a shift in the temperature at which performance is optimized [1,9].

When organisms thermoregulate, the covariance between organismal body temperature and environmental temperature is diminished [10–13]. For ectotherms, this lack of correspondence between body temperature and environmental temperature occurs primarily because individuals selectively use a narrow range of suitable microclimates within a thermally heterogeneous habitat [14]. Individuals that behaviourally thermoregulate are unlikely to experience thermal extremes and may, therefore, buffer themselves from selection on thermal physiology, a phenomenon known as the ‘Bogert Effect’ [15–17]. Indeed, studies comparing thermoregulating species distributed across elevational or latitudinal gradients often find that thermal physiological traits are invariant despite large differences in local environmental temperatures [18,19]. In the context of climate change, this suggests that behavioural compensation for changing thermal environments can actually increase extinction risk over the long term by inhibiting evolutionary adaptation in thermal physiology [7,18]. Additionally, thermoregulatory behaviour itself may evolve. For example, climate change may favour individuals that are more efficient at locating preferred microclimates, or those that spend more time thermoregulating relative to foraging and other activities. Thus, evolutionary change in thermoregulatory behaviour may help maintain population viability even while constraining evolutionary shifts in thermal physiology.

Previous studies have shown that both thermal physiology and thermoregulatory behaviour can be targets of selection in wild populations of small ectotherms, including lizards [7,20,21], insects [22] and molluscs [23]. Using a field experiment, Logan *et al.* [7] demonstrated that brown anole lizards (*Anolis sagrei*) transplanted from an interior forest habitat to a warmer and more thermally variable open habitat experienced strong directional selection for higher thermal optima and broader performance breadths. Similarly, Gilbert & Miles [20] documented selection on thermoregulatory behaviour in a population of ornate tree lizards (*Urosaurus ornatus*), in which individuals that preferred higher body temperatures in a laboratory thermal gradient were more likely to survive in nature. However, selection will only result in evolution if phenotypic variation has an additive genetic basis [4,24,25]. To date, there are very few studies on the heritability of thermal traits in animals. Pioneering studies that reported broad-sense heritability in insects have suggested that thermal traits may have an additive genetic basis [26–28], but to our knowledge, no study has quantified the narrow-sense heritability of a thermal performance curve in any species, and only two studies [29,30] have estimated broad- or narrow-sense heritability for aspects of thermoregulatory behaviour.

For this study, we collected adult brown anoles from two islands in The Bahamas at sites differing dramatically in their thermal characteristics. Lizards from the island of Great Exuma come from densely vegetated habitat with abundant shade and have experienced a relatively cool, thermally stable environment, whereas lizards from Eleuthera occupy an exposed, sparsely vegetated peninsula and have experienced warmer, more thermally variable conditions (table 1, figure S1). Previously, Logan *et al.* [7] measured natural selection on components of the thermal performance curve for

sprint speed in these populations. In the cooler and more thermally stable environment on Great Exuma, the thermal performance curve was not under selection (table 1). However, when lizards from an interior forest environment on Eleuthera (similar in its thermal properties to the environment on Great Exuma) were transplanted to the warmer and more thermally variable peninsula, they underwent strong viability selection for higher thermal optima and broader performance breadths (table 1). Eleuthera parents for the current breeding experiment were captured just prior to this transplant study, whereas Exuma parents were captured just after that study but at an adjacent site with similar habitat. Thus, our breeding populations would have presumably experienced different local thermal environments for many generations. To test the prediction that Great Exuma and Eleuthera populations have genetically diverged in response to their contrasting thermal environments in ways congruent with previous estimates of selection, we bred adults from these populations in a paternal half-sibling design and raised their offspring in a laboratory common garden environment, then assayed F_1 offspring for thermal performance curves and aspects of thermoregulatory behaviour. Next, we analysed pedigrees for each population to determine whether thermal performance curves and thermoregulatory behaviour have an additive genetic basis such that they can respond rapidly to selection imposed by future climate change.

2. Material and methods

(a) Common garden and breeding design

We collected adult brown anoles (*A. sagrei*) from each of two islands in The Bahamas (Great Exuma: 23°29' N, 75°45' W; Eleuthera: 24°50' N, 76°19' W). To reduce the potential for fertilization using stored sperm [31], we collected adults between 25 and 30 January 2012, prior to the onset of the breeding season (April to October). Individuals were transported to a breeding facility at the University of Virginia, where they served as the parental generation for our breeding experiment. We maintained adults individually in plastic cages (males: 40 × 23 × 32 cm; females: 30 × 20 × 20 cm; Lee's Kritter Keeper, San Marcos, CA) containing a potted plant (*Coleus*) for oviposition, a carpet substrate, a section of PVC pipe for perching and hiding (30 cm length, 2.5 cm diameter), and a strip of fibreglass screen suspended across the top of the cage for basking. We placed cages directly beneath two ReptiSun 10.0 UVB bulbs (ZooMed, San Luis Obispo, CA) and housed them at constant temperature (29°C diurnal, 25°C nocturnal), relative humidity (65%) and photoperiod (12 L:12D during simulated winter, 13 L:11D during the breeding season). Three times per week, we offered adult males 5–7 large (1/2 inch = 12.7 mm) crickets (*Gryllus assimilis* and *Gryllodes sigillatus*; Ghann's Cricket Farm, Augusta, GA), and adult females 3–5 medium (3/8 inch = 9.5 mm) crickets. We dusted crickets weekly with Fluker's Reptile Vitamin and Calcium supplements (Fluker's Cricket Farms, Port Allen, LA) and sprayed cage walls and potted plants twice daily with deionized water.

We paired each sire with two dams from the same population, such that each female mated with only one male. This design allowed us to estimate genetic variances using the phenotypes of full-sib and paternal half-sib F_1 progeny. After isolating each female for two to three months following capture to ensure that they were not producing eggs from stored sperm, we introduced females into the cages of males and allowed them to mate for 14 days before returning them to their original cages. Previous

Table 1. A comparison of habitat structure, thermal environments, lizard body temperatures and selection gradients for components of the thermal performance curve (TPC) measured at locations near the source populations for this common garden breeding experiment. These data are presented in depth by Logan *et al.* [7].

	Exuma	Eleuthera
habitat	relatively dense vegetation, closed canopy	relatively sparse vegetation, open canopy
operative environmental temperatures (T_e)		
mean daily T_e	29.8 ± 0.24 s.e.m. $^{\circ}\text{C}$	32.5 ± 0.21 s.e.m. $^{\circ}\text{C}$
max daily T_e	32.1 ± 0.46 s.e.m. $^{\circ}\text{C}$	36.2 ± 0.39 s.e.m. $^{\circ}\text{C}$
weekly range in mean daily T_e	28.9–31.1 $^{\circ}\text{C}$	28.4–35.2 $^{\circ}\text{C}$
seasonal variance in mean weekly T_e	0.5 $^{\circ}\text{C}$	2.9 $^{\circ}\text{C}$
summary of T_e	cooler and more thermally stable	warmer and more thermally variable
lizard field-active body temperatures (T_b)		
mean T_b	32.9 ± 0.24 s.e.m. $^{\circ}\text{C}$	34.4 ± 0.21 s.e.m. $^{\circ}\text{C}$
maximum T_b	36.4 $^{\circ}\text{C}$	38.6 $^{\circ}\text{C}$
summary of T_b	lower field-active body temperatures	higher field-active body temperatures
selection gradients (β) on TPC parameters		
maximal performance (P_{max})	0.23 ± 0.15 s.e.m.	0.19 ± 0.23 s.e.m.
thermal optimum (T_{opt})	0.02 ± 0.13 s.e.m.	0.56 ± 0.23 s.e.m.*
performance breadth (T_{br})	0.13 ± 0.14 s.e.m.	0.49 ± 0.20 s.e.m.*
summary of natural selection	no linear selection on TPC parameters	selection for higher T_{opt} and broader T_{br}

*Significant selection gradients ($p < 0.05$) are indicated.

studies have shown that, even when females are collected from the wild at the peak of the breeding season, they shift to the fertilization of eggs with sperm from captive mates within an average time of less than three weeks [31]. To generate paternal half-siblings, we introduced a second female into the cage of each male a week after the removal of the first female, and again allowed the pair to mate for 14 days. Four months after initial mating, we paired the same individuals a second time to provide females with additional sperm. The following year, we repeated the same breeding protocol with the same sire-dam pairs to increase the number of offspring in each family.

Each week, we checked each female's potted plant for new eggs, which we transplanted to individual plastic containers containing moist vermiculite (1 : 1 deionized water to vermiculite by mass). Egg containers were then placed in a Percival Intellus 136VL incubator set to maintain 28 $^{\circ}\text{C}$, 80% relative humidity, and a 12 L : 12D photoperiod. We checked egg containers twice daily for new hatchlings, which we assigned a unique toe-clip for permanent identification and housed individually in cages identical to those of adult females. We offered each hatchling 10–15 pinhead crickets (*Acheta domestica*) daily, dusted with Fluker's Reptile Vitamin and Calcium supplements. At three months of age, we began offering each lizard 2–3 small (1/4 inch = 6.4 mm) crickets (*G. sigillatus*) three times per week (dusted weekly). At 12 months of age, we began feeding each lizard 5–7 large (1/2 inch = 12.7 mm) crickets (*G. sigillatus*) three times per week (dusted weekly). We waited until offspring had grown to adulthood (minimum 18 months of age) before phenotyping them for thermal performance curves and thermoregulatory behaviour. Because we had bred the parental generation in two successive years, we phenotyped the resulting F_1 cohorts in two separate years, but at similar body sizes.

(b) Thermal performance curves

We estimated the thermal sensitivity of sprint speed in 289 and 119 adult male F_1 offspring from Great Exuma (48 sires, 85 dams) and Eleuthera (35 sires, 56 dams) parents, respectively.

We heated or cooled lizards to each of six temperatures (15, 21, 27, 32, 37 and 42 $^{\circ}\text{C}$, in a randomized order) in a Percival Intellus 136VL incubator. Each day, lizards were phenotyped at two temperatures with a minimum of a 2 h break between trials ('trial' = a set of runs at a given temperature). Each individual was encouraged to run along a wooden dowel rod (1 m long, 2.5 cm in diameter) positioned at a 20 $^{\circ}$ angle to prevent hopping. The dowel was demarcated every 10 cm and trials were filmed with a fast-frame (60 frames per second (fps)) digital video camera. Each lizard was run three times in quick succession at each temperature, and body temperatures were confirmed at the start and end of each trial using a cloacal thermometer. Runs that occurred more than 30 s after the lizard had been removed from the incubator were not included in subsequent analyses.

Sprint videos were analysed in the motion-analysis software program Kinovea (www.kinovea.org). Sprint speed was calculated using the frame-rate of the camera, and we recorded the fastest sprint speed during each trial over any 10 cm segment [7,32]. If the lizard could not run at least 10 cm at the coolest and warmest temperatures, we scored that trial as a speed of zero [7,32]. At the intermediate temperatures, if the lizard fell off the dowel rod, hopped, or could not maintain coordination for at least 10 cm, we discarded the trial and did not include it in further analyses [7,32]. If these events occurred at the extreme temperatures (15 $^{\circ}\text{C}$ and 42 $^{\circ}\text{C}$), they were likely a result of temperature stress and were scored as a sprint speed of zero.

We generated thermal performance curves following [7], fitting the full set of asymmetric parabolic functions built into the software program TableCurve 2.0 to the raw sprint data [7,32–35]. We fitted both zero and non-zero intercept versions of each function to each individual. We then chose the best curve fit for each individual using AIC (if the curves differed in AIC score by two or greater, we considered them significantly different). If AIC could not distinguish between the best curves, we chose the curve with the fewest parameters. If two curves did not differ in AIC score or number of parameters, we chose the curve with the highest r^2 value. By solving each individual's curve at 0.1 $^{\circ}\text{C}$ intervals, we extracted three values which describe

the general shape of thermal performance curves: the thermal optimum (body temperature at which performance is greatest, T_{opt}), the maximal performance across all temperatures (P_{max}), and the performance breadth (the range of body temperatures at which the lizard achieves at least 80% of maximal performance, T_{br}) [1].

We compared sprint speed at each temperature between Great Exuma and Eleuthera populations using restricted maximum-likelihood mixed models that included 'run order' (first, second, or third sprint at a given temperature) and 'population' (Great Exuma or Eleuthera) as fixed effects with interactions, as well as 'individual' as a random effect (to account for repeated measures). Additionally, we compared the means of individual estimates of thermal optima, performance breadths, and maximal performances between populations using ANOVA. All comparisons of thermal performance curves were conducted in JMP Pro 13 (SAS Institute, Cary, NC, USA). Because we did not measure lizards from Eleuthera in the second year of our study, all comparisons between Eleuthera and Exuma are based on data from the first year only.

(c) Thermoregulatory behaviour

We quantified variation in thermoregulatory behaviour for 156 F_1 adult male brown anoles produced from Great Exuma parents (44 sires, 71 dams) using a laboratory thermal gradient. Each lane of our thermal gradient was 1.2 m long by 0.25 m wide by 0.35 m tall. We fixed a 60 W incandescent indoor floodlight bulb above one end of each lane and put six frozen gel packs under the other end. This generated a thermal gradient ranging from 20 to 48°C. We taped 40 gauge, type T thermocouples to the cloacal surface of each individual prior to introducing them to the gradient, then allowed each individual to acclimate to the gradient for a period of 10 min prior to taking body temperature measurements. The thermocouples were connected to an OMEGA eight-channel data acquisition module (TC-08, OMEGA Engineering, Inc., Norwalk, CT, USA), which recorded body temperatures every 10 s for 90 min. The initial body temperature of each lizard when introduced to the gradient was approximately 30°C. We quantified the mean, median, minimum, maximum and range of body temperatures achieved in the thermal gradient as different estimates of thermoregulatory behaviour for each individual. The mean and median body temperatures chosen in a gradient can be thought of as estimates of an individual's thermal preference, whereas the minimum, maximum and range of body temperatures chosen in a gradient can collectively be thought of as estimates of an individual's thermoregulatory precision, its desire to remain at a narrow versus broad range of temperatures, or its tendency to endure very high or low body temperatures.

(d) Quantitative genetic analyses

We employed restricted maximum-likelihood 'animal models' in the software program ASReml 4 (VSN International Limited, Hemel Hempstead, UK) to estimate the additive genetic variances and heritabilities of the thermal sensitivity of sprint speed (thermal performance curves) and thermoregulatory behaviour in our study populations. All parameters were estimated separately for each population. For thermal sensitivity of sprint speed, we analysed the data in two ways. First, we analysed sprint speed at each trial temperature in separate animal models. Each model included run (first, second or third sprint for each individual at each test temperature) and year (2012 or 2013 cohort, Great Exuma only) as fixed effects, a pedigree-linked random-effect of individual to estimate additive genetic variances, and a second random-effect of individual to estimate permanent environmental effects. Second, after verifying normality, we estimated variance components for the composite traits (thermal optimum, maximal performance and performance

breadth) that describe the shape of the thermal performance curve. We did not conduct more detailed multivariate or function-valued analyses due to low additive genetic variances detected during univariate analyses (see Results). Models for these composite traits included a fixed-effect of year (2012 or 2013 cohort, Great Exuma only) and a pedigree-linked random-effect of individual. Finally, we implemented animal models in ASReml for each aspect of thermoregulatory behaviour (mean, median, minimum, maximum and range of body temperatures chosen in a thermal gradient).

To determine statistical significance of variance components, we used likelihood-ratio tests [36]. Significance of additive genetic variance was determined by comparing a full model to one in which genetic variance was fixed at zero (1 df), and significance of permanent environmental variance was tested by comparing a model that included both permanent environmental variance and residual variance to one that included only residual variance (1 df). For all models, we calculated heritability and repeatability (where appropriate) and their standard errors (in ASReml) by dividing the additive genetic variance or the total individual variance (additive genetic variance plus permanent environmental variance) by the total phenotypic variance. We did not estimate genetic covariances due to the low additive genetic variance underlying most traits (see Results).

3. Results

(a) Thermal performance curves

Thermal performance curves differed between laboratory-reared offspring from each of our study populations (figure 1). Eleuthera lizards sprinted faster than Great Exuma lizards at 15°C ($F_{1,310} = 17.53$, $p < 0.0001$). By contrast, Great Exuma lizards outperformed those from Eleuthera at 21°C ($F_{1,293} = 7.28$, $p = 0.0074$), 27°C ($F_{1,299} = 59.65$, $p < 0.0001$), 32°C ($F_{1,306} = 104.40$, $p < 0.0001$), 37°C ($F_{1,307} = 18.03$, $p < 0.0001$) and 42°C ($F_{1,310} = 6.76$, $p = 0.0098$). Additionally, lizards from Great Exuma had greater maximal performance (Great Exuma mean = 0.97 m s^{-1} , Eleuthera mean = 0.84 m s^{-1} ; $F_{1,268} = 21.93$, $p < 0.0001$) and broader performance breadths (Great Exuma mean = 10.0°C, Eleuthera mean = 8.8°C; $F_{1,268} = 13.99$, $p = 0.0002$) than those from the Eleuthera population, whereas Eleuthera lizards had a higher thermal optimum (Great Exuma mean = 34.7°C, Eleuthera mean = 36.0°C; $F_{1,268} = 12.97$, $p = 0.0004$; figure 2).

When we evaluated sprint speeds at each test temperature as independent traits, most were not significantly heritable ($0 < h^2 < 0.06$) for either population (table 2). However, for Great Exuma, sprint speed was significantly heritable at 37°C ($h^2 = 0.124 \pm 0.077$ s.e.m.), and for Eleuthera, sprint speed was significantly heritable at 15°C ($h^2 = 0.488 \pm 0.057$ s.e.m.). Sprint speed was repeatable at every temperature in both populations (significant effect of 'individual'; lowest and highest r^2 values, respectively, were 0.283 and 0.579; mean $r^2 = 0.477$; table 2). The composite traits that describe the shape of the thermal performance curve (maximal performance, performance breadth and the thermal optimum) were not significantly heritable for either population (table 2).

(b) Thermoregulatory behaviour

The mean, median, minimum, maximum and range of body temperatures selected by Great Exuma lizards in a laboratory thermal gradient were $32.7^\circ\text{C} \pm 0.34$ s.e.m., $32.4^\circ\text{C} \pm 0.35$

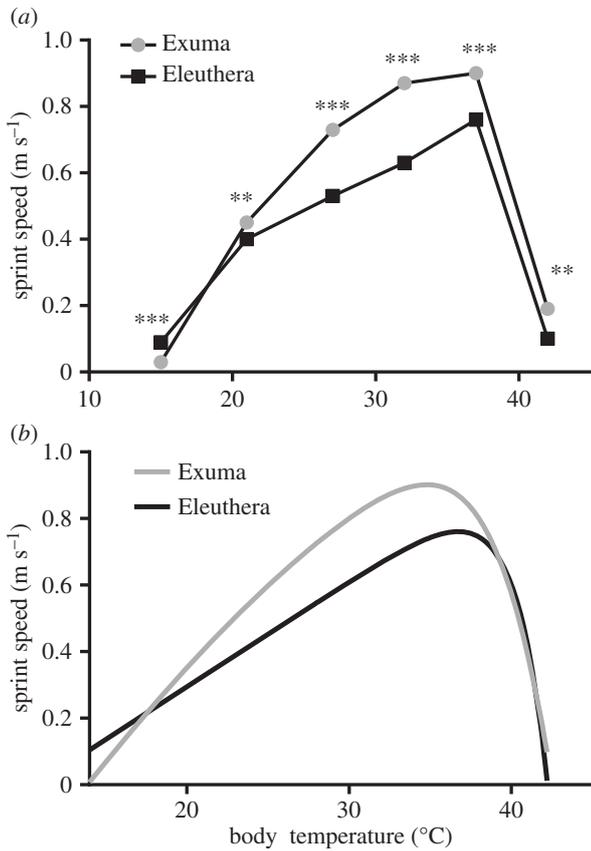


Figure 1. Thermal performance curves differed between laboratory-reared populations. Exuma individuals performed better across all test temperatures except 15°C, where Eleuthera individuals performed better. (a) Standard errors are too small to be visible. (b) The best-fit curves from which the composite traits (thermal optimum, performance breadth and maximal performance) were extracted. Two and three asterisks denote significance to the thousandths (less than 0.009) and ten-thousandths (less than 0.0009) decimal places, respectively.

s.e.m., 29.2°C ± 0.33 s.e.m., 36.6°C ± 0.45 s.e.m. and 7.36°C ± 0.44 s.e.m., respectively. Of these traits, only the range was significantly heritable ($h^2 = 0.41 \pm 0.21$ s.e.m.; table 2).

4. Discussion

Consistent with the hypothesis that thermal performance curves have genetically diverged in contrasting thermal environments, captive-bred F_1 offspring from our two source populations had thermal performance curves that differed in several characteristics. Specifically, they differed in performance capacity at every body temperature measured (figure 1), as well as in the composite variables that describe the shape of the thermal performance curve (figure 2). Nevertheless, we did not detect significant heritability underlying most of the thermal physiology and thermoregulatory behaviour traits we measured, suggesting that these traits lack sufficient additive genetic variance to respond rapidly to contemporary selection.

Differences in thermal performance curves between our two laboratory-reared populations were only partially consistent with the contrasting selection gradients measured in nature. For example, Logan *et al.* [7] found that anoles from a thermally benign environment near our source population on Great Exuma did not experience natural selection on their thermal performance curves (table 1). By contrast,

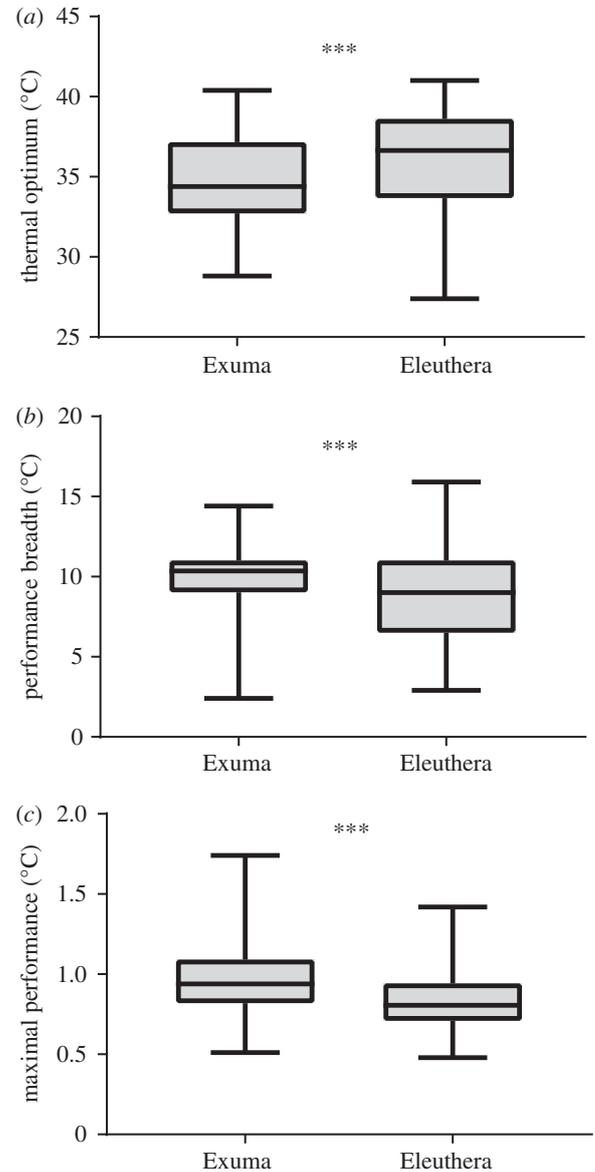


Figure 2. The composite traits that describe the shape of the thermal performance curve differ among study populations. The Exuma population had a lower thermal optimum (a), a higher performance breadth (b) and a higher maximal performance (c). Boxes and whiskers range from the 25th to the 75th quartiles, and 10th to 90th percentiles, respectively. The line passing through the box represents the median. Three asterisks denote significance to the ten-thousandths ($p < 0.0009$) decimal place.

anoles from a cool interior location on Eleuthera that were transplanted to the hotter and more thermally variable peninsula from which we sampled our source population experienced higher mean body temperatures and underwent directional selection favouring a higher thermal optimum and a broader performance breadth [7]. Thus, the parental generations for our breeding colonies should have experienced a history of selection similar to those described above and summarized in table 1. Consistent with these previous field estimates of selection, Eleuthera offspring raised in a laboratory common garden environment had a higher thermal optimum for sprint speed. Conversely, Eleuthera offspring raised in the laboratory had narrower performance breadths, whereas previous field measurements revealed directional selection for broader performance breadths following transplantation to this site. It should also be noted that F_1 offspring from Eleuthera generally exhibited lower

Table 2. Variance components (additive genetic = V_A ; permanent environmental = V_{PE} ; residual = V_E), heritabilities (h^2) and repeatabilities (r^2), with s.e. in parentheses, for running speed (v) at six body temperatures and for the composite variables describing the shape of the thermal performance curve (thermal optimum = T_{opt} ; maximal performance = P_{max} ; performance breadth = T_{br}). For clarity, variance components (and their s.e.) were multiplied by 10^3 for all traits except T_{opt} and T_{br} . Statistical significance ($p < 0.05$) of variance components (as determined via likelihood-ratio tests) is indicated in italics.

population	trait	V_A	V_{PE}	V_E	h^2	r^2
Exuma	v15°C	0.51 (0.53)	2.39 (0.62)	6.04 (0.36)	0.057 (0.059)	0.324 (0.038)
	v21°C	0	15.52 (1.71)	12.10 (0.76)	0	0.562 (0.033)
	v27°C	2.00 (3.28)	27.29 (4.28)	21.31 (1.31)	0.040 (0.065)	0.579 (0.032)
	v32°C	0	36.15 (4.03)	29.80 (1.86)	0	0.548 (0.034)
	v37°C	8.70 (5.54)	27.70 (5.78)	33.95 (2.03)	0.124 (0.077)	0.517 (0.034)
	v42°C	0.56 (5.57)	55.44 (8.24)	48.33 (2.86)	0.005 (0.054)	0.537 (0.033)
	T_{opt}	0.10 (0.76)	n.a.	7.50 (10.08)	0.013 (0.100)	n.a.
	P_{max}	0	n.a.	53.14 (4.84)	0	n.a.
	T_{br}	0.74 (0.81)	n.a.	6.38 (0.94)	0.104 (0.113)	n.a.
Eleuthera	v15°C	7.19 (1.38)	0	7.53 (0.69)	0.488 (0.057)	0.488 (0.057)
	v21°C	1.44 (3.70)	10.56 (4.08)	11.98 (1.26)	0.060 (0.153)	0.501 (0.060)
	v27°C	0	14.34 (2.87)	19.38 (1.86)	0	0.425 (0.059)
	v32°C	0	16.15 (3.07)	18.84 (1.80)	0	0.462 (0.057)
	v37°C	0	29.11 (5.18)	28.85 (2.69)	0	0.502 (0.054)
	v42°C	0	11.29 (2.87)	28.61 (2.66)	0	0.283 (0.060)
	T_{opt}	0	n.a.	10.41 (1.50)	0	n.a.
	P_{max}	0	n.a.	35.7 (5.12)	0	n.a.
	T_{br}	0	n.a.	8.44 (1.21)	0	n.a.

sprint speeds across all temperatures relative to those from Great Exuma, such that overall differences in performance between populations may be driving differences in thermal performance curves in a way that is independent from thermal adaptation per se. It is also possible that differing behavioural responses to the laboratory environment underlie differences in sprint performance and that this, again, is independent of thermal adaptation. Lastly, we cannot rule out a role for maternal effects given that our assays were conducted on F_1 individuals from wild-caught parents [37].

Although our common garden experiment suggests that thermal performance curves have diverged genetically between these populations, we found little evidence of additive genetic variance underlying the thermal sensitivity of sprint speed. At five of the six temperatures in our study, we did not detect significant heritability for sprint speed in either population, nor did we detect heritability for the composite traits (thermal optimum, performance breadth, maximal performance) that define the shape of the thermal performance curve. Although these composite traits are difficult to measure with precision and resultant noise in those data may have reduced our capacity to detect heritability, sprint speed was repeatable at each test temperature in both populations, suggesting that it should have been possible to detect underlying additive genetic variance if it was present at moderate magnitudes. Moreover, using the same F_1 individuals that we included in this study, Cox *et al.* [36,38] previously detected significant additive genetic variance for body size, dewlap size and dewlap colouration, as well as for composite traits such as growth trajectories. One interpretation for the lack of additive genetic variance in the present study is that strong selection in the past has led

to divergence between populations while simultaneously eroding genetic variance for these traits.

As with our analyses of thermal performance curves, four of the five behavioural thermoregulation traits that we measured were not heritable, including the mean temperature achieved in a thermal gradient (an ecologically important trait that is usually taken to approximate the 'preferred temperature' of an individual) [20,39,40]. In the context of climate change, thermoregulatory behaviour is usually only considered in its capacity to constrain the evolution of thermal tolerance (by reducing the range of environmental states experienced by individuals) [15,18], or in its ability to mitigate the negative impacts of warming by allowing individuals to seek out thermal refugia [12,41,42]. Here, we tested the idea that thermoregulatory behaviour itself may have the capacity to evolve rapidly, an idea that has received very little attention in the literature (but see [29], which also found no detectable additive genetic variance underlying one aspect of thermoregulatory behaviour).

Gilbert & Miles [20] previously documented strong natural selection favouring higher thermal preferences in a wild population of ornate tree lizards (*U. ornatus*) from Arizona. By contrast to the brown anoles studied by Logan *et al.* [7], the tree lizard population was not transplanted to a novel thermal environment, suggesting that selection on thermoregulatory behaviour may typically be strong even when environments do not change. Thus, selection may be particularly likely to erode genetic variance in traits related to thermoregulatory behaviour (because selection should be even stronger when environments change). Nevertheless, it should be noted that we did detect relatively high heritability for the range of body temperatures achieved in a thermal

gradient, and our estimate of heritability for maximum body temperature approached significance (table 3). With higher sample sizes, therefore, we might have detected non-zero heritability for several aspects of thermoregulatory behaviour, especially those that are associated with thermoregulatory precision.

The lack of genetic variance underlying thermal physiology and thermoregulatory behaviour in brown anoles suggest that the most likely avenue of rapid, short-term change in this trait is phenotypic plasticity [43]. Indeed, brown anoles are invasive over large portions of the tropical and subtropical Western hemisphere and have shown little difficulty adjusting to novel thermal environments [44], suggesting that rapid genetic adaptation may be unnecessary for their continued survival under climate change. Nevertheless, the capacity for terrestrial ectotherms in general to respond to environmental change through plasticity may be limited [45], and the importance of acclimatization is likely to be idiosyncratic and species-specific. Further work on diverse taxa is needed to understand the extent to which thermal performance curves and thermoregulatory behaviour may be evolutionarily fixed in the short-term, and the extent to which plasticity can reduce extinction probabilities. Moreover, the relative importance of evolutionary change in thermal physiology versus thermoregulatory behaviour is a promising area of future research [46].

Although we found that two laboratory-reared populations of brown anoles from divergent thermal environments experiencing contrasting patterns of selection differ in their thermal performance curves, we also found that neither population appears to have sufficient standing genetic variation to evolve rapidly in response to climate change. The extent to which this conclusion can be generalized to other species, or beyond the laboratory common garden environment in which we measured heritability, remains to be seen, but further research is clearly needed. Recent studies (including our own; [7,20,32]) have emphasized the potential role of evolutionary rescue in the face of climate change. Nevertheless, our results suggest that, in at least in some species, other avenues of resilience such as behavioural

Table 3. Variance components (additive genetic = V_A ; residual = V_E) and heritabilities (h^2), with s.e. in parentheses, for thermoregulatory behaviour (mean, median, minimum, maximum and range of body temperatures achieved in a laboratory thermal gradient). Statistical significance ($p < 0.05$) of additive genetic variance (as determined via likelihood-ratio tests) is indicated in italics.

trait	V_A	V_E	h^2
mean	2.16 (3.08)	15.85 (3.40)	0.12 (0.17)
median	1.98 (3.24)	17.20 (3.61)	0.10 (0.17)
minimum	0.08 (2.97)	16.00 (3.35)	0.07 (0.17)
maximum	7.41 (6.56)	25.71 (6.53)	0.22 (0.19)
range	<i>13.20 (7.46)</i>	18.73 (6.46)	0.41 (0.21)

adjustments, plasticity and range shifts may be more important.

Ethics. This study followed all relevant ethical guidelines and was approved by the University of Virginia Animal Care and Use Committee (protocol 3896).

Data accessibility. Data supporting this manuscript are provided in the online electronic supplementary material.

Authors' contributions. M.L.L. designed the study, collected and analysed data and wrote the first manuscript draft; J.D.C. collected and analysed data and helped revise the manuscript; A.L.G. collected data and helped revise the manuscript; D.B.M. contributed to study design, collected and analysed data and helped revise the manuscript; A.K.C. analysed data and helped revise the manuscript; J.W.M. contributed to study design, analysed data and helped write the manuscript; R.M.C. raised the lizard colonies, contributed to study design and helped write the manuscript. All authors gave final approval for publication.

Competing interests. We have no competing interests.

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References

- Angilletta MJ. 2009 *Thermal adaptation*, p. 320. Oxford, UK: Oxford University Press.
- Sinervo B *et al.* 2010 Erosion of lizard diversity by climate change and altered thermal niches. *Science* **328**, 894–899. (doi:10.1126/science.1184695)
- Hoffmann AA. 2010 Physiological climatic limits in *Drosophila*: patterns and implications. *J. Exp. Biol.* **213**, 870–880. (doi:10.1242/jeb.037630)
- Hoffmann AA, Sgro CM. 2011 Climate change and evolutionary adaptation. *Nature* **470**, 479–485. (doi:10.1038/nature09670)
- Huey RB, Kingsolver JG. 1989 Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**, 131–135. (doi:10.1016/0169-5347(89)90211-5)
- Angilletta MJ, Wilson RS, Navas CA, James RS. 2003 Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* **18**, 234–240. (doi:10.1016/S0169-5347(03)00087-9)
- Logan ML, Cox RM, Calsbeek R. 2014 Natural selection on thermal performance in a novel thermal environment. *Proc. Natl Acad. Sci. USA* **111**, 14 165–14 169. (doi:10.1073/pnas.1404885111)
- Gilchrist GW. 1995 Specialists and generalists in changing environments 1. Fitness landscapes of thermal sensitivity. *Am. Nat.* **146**, 252–270. (doi:10.1086/285797)
- Angilletta MJ, Niewiarowski PH, Navas CA. 2002 The evolution of thermal physiology in ectotherms. *J. Therm. Biol.* **27**, 249–268. (doi:10.1016/S0306-4565(01)00094-8)
- Sears MW, Angilletta MJ, Schuler MS, Borchert J, Dilliplane KF, Stegman M, Rusch TW, Mitchell WA. 2016 Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proc. Natl Acad. Sci. USA* **113**, 10 595–10 600. (doi:10.1073/pnas.1604824113)
- Sears MW, Raskin E, Angilletta MJ. 2011 The world is not flat: defining relevant thermal landscapes in the context of climate change. *Integr. Comp. Biol.* **51**, 666–675. (doi:10.1093/icb/1111)
- Kearney M, Shine R, Porter WP. 2009 The potential for behavioral thermoregulation to buffer 'cold-blooded' animals against climate warming. *Proc. Natl Acad. Sci. USA* **106**, 3835–3840. (doi:10.1073/pnas.0808913106)
- Cox CL *et al.* 2018 Do ring-necked snakes choose retreat sites based upon thermal preferences? *J. Therm. Biol.* **71**, 232–236. (doi:10.1016/j.jtherbio.2017.11.020)
- Clusella-Trullas S, Chown SL. 2011 Comment on 'Erosion of lizard diversity by climate change and altered thermal niches'. *Science* **332**, 1. (doi:10.1126/science.1195193)
- Huey RB, Hertz PE, Sinervo B. 2003 Behavioral drive versus behavioral inertia in evolution: a null model

- approach. *Am. Nat.* **161**, 357–366. (doi:10.1086/346135)
16. Munoz MM, Losos JB. 2018 Thermoregulatory behavior simultaneously promotes and forestalls evolution in a tropical lizard. *Am. Nat.* **191**, E15–E26. (doi:10.1086/694779)
 17. Munoz MM, Stimola MA, Algar AC, Conover A, Rodriguez AJ, Landestoy MA, Bakken GS, Losos JB. 2014 Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proc. R. Soc. B* **281**, 20132433. (doi:10.1098/rspb.2013.2433)
 18. Buckley LB, Ehrenberger JC, Angilletta MJ. 2015 Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. *Funct. Ecol.* **29**, 1038–1047. (doi:10.1111/1365-2435.12406)
 19. Buckley LB, Urban MC, Angilletta MJ, Crozier LG, Rissler LJ, Sears MW. 2010 Can mechanism inform species' distribution models? *Ecol. Lett.* **13**, 1041–1054. (doi:10.1111/j.1461-0248.2010.01479.x)
 20. Gilbert AL, Miles DB. 2017 Natural selection on thermal preference, critical thermal maxima and locomotor performance. *Proc. R. Soc. B* **284**, 20170536. (doi:10.1098/rspb.2017.0536)
 21. Artacho P, Saravia J, Ferrandiere BD, Perret S, Galliard JF. 2015 Quantification of correlational selection on thermal physiology, thermoregulatory behavior, and energy metabolism in lizards. *Ecol. Evol.* **5**, 3600–3609. (doi:10.1002/ece3.1548)
 22. Kingsolver JG, Gomulkiewicz R. 2003 Environmental variation and selection on performance curves. *Integr. Comp. Biol.* **43**, 470–477. (doi:10.1093/icb/43.3.470)
 23. Artacho P, Nespola RF. 2009 Natural selection reduces energy metabolism in the garden snail, *Helix aspersa* (*Cornu aspersum*). *Evolution* **63**, 1044–1050. (doi:10.1111/j.1558-5646.2008.00603.x)
 24. van Heerwaarden B, Sgro CM. 2014 Is adaptation to climate change really constrained in niche specialists? *Proc. R. Soc. B* **281**, 20140396. (doi:10.1098/rspb.2014.0396)
 25. Lande R, Arnold SJ. 1983 The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226. (doi:10.2307/2408842)
 26. Gilchrist GW. 1996 A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* **50**, 1560–1572. (doi:10.2307/2410892)
 27. Kingsolver JG, Ragland GJ, Shlichta JG. 2004 Quantitative genetics of continuous reaction norms: thermal sensitivity of caterpillar growth rates. *Evolution* **58**, 1521–1529. (doi:10.1111/j.0014-3820.2004.tb01732.x)
 28. Latimer CAL, Wilson RS, Chenoweth SF. 2011 Quantitative genetic variation for thermal performance curves within and among natural populations of *Drosophila serrata*. *J. Evol. Biol.* **24**, 965–975. (doi:10.1111/j.1420-9101.2011.02227.x)
 29. Paranjpe DA, Bastiaans E, Patten A, Cooper RD, Sinervo B. 2013 Evidence of maternal effects on temperature preference in side-blotched lizards: implications for evolutionary response to climate change. *Ecol. Evol.* **3**, 1977–1991. (doi:10.1002/ece3.614)
 30. Bestion E, Clobert J, Cote J. 2015 Dispersal response to climate change: scaling down to intraspecific variation. *Ecol. Lett.* **18**, 1226–1233. (doi:10.1111/ele.12502)
 31. Calsbeek R, Bonneaud C, Prabhu S, Manoukis N, Smith TB. 2007 Multiple paternity and sperm storage lead to increased genetic diversity in *Anolis* lizards. *Evol. Ecol. Res.* **9**, 495–503.
 32. Logan ML, Duryea MC, Molnar OR, Kessler BJ, Calsbeek R. 2016 Spatial variation in climate mediates gene flow across an island archipelago. *Evolution* **70**, 2395–2403. (doi:10.1111/evo.13031)
 33. Angilletta MJ. 2006 Estimating and comparing thermal performance curves. *J. Therm. Biol.* **31**, 541–545. (doi:10.1016/j.jtherbio.2006.06.002)
 34. Logan ML, Fernandez SG, Calsbeek R. 2015 Abiotic constraints on the activity of tropical lizards. *Funct. Ecol.* **29**, 694–700. (doi:10.1111/1365-2435.12379)
 35. Logan ML, Huynh RK, Precious RA, Calsbeek RG. 2013 The impact of climate change measured at relevant spatial scales: new hope for tropical lizards. *Glob. Change Biol.* **19**, 3093–3102. (doi:10.1111/gcb.12253)
 36. Cox RM, Costello RA, Camber BE, McGlothlin JW. 2017 Multivariate genetic architecture of the *Anolis* dewlap reveals both shared and sex-specific features of a sexually dimorphic ornament. *J. Evol. Biol.* **30**, 1262–1275. (doi:10.1111/jeb.13080)
 37. Warner DA. 2014 Fitness consequences of maternal and embryonic responses to environmental variation: using reptiles as models for studies of developmental plasticity. *Integr. Comp. Biol.* **54**, 757–773. (doi:10.1093/icb/ucu099)
 38. Cox RM, Cox CL, McGlothlin JW, Card DC, Andrew AL, Castoe TA. 2017 Hormonally mediated increases in sex-biased gene expression accompany the breakdown of between-sex genetic correlations in a sexually dimorphic lizard. *Am. Nat.* **189**, 315–332. (doi:10.1086/690105)
 39. Clusella-Trullas S, Terblanche JS, van Wyk JH, Spotila JR. 2007 Low repeatability of preferred body temperature in four species of Cordylid lizards: temporal variation and implications for adaptive significance. *Evol. Ecol.* **21**, 63–79. (doi:10.1007/s10682-006-9124-x)
 40. Hertz PE, Huey RB, Stevenson RD. 1993 Evaluating temperature regulation by field-active ectotherms: the fallacy of the inappropriate question. *Am. Nat.* **142**, 796–818. (doi:10.1086/285573)
 41. Buckley LB, Tewksbury JJ, Deutsch CA. 2013 Can terrestrial ectotherms escape the heat of climate change by moving? *Proc. R. Soc. B* **280**, 20131149. (doi:10.1098/rspb.2013.1149)
 42. Huey RB, Kearney MR, Krockenberger A, Holtum JAM, Jess M, Williams SE. 2012 Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. B* **367**, 1665–1679. (doi:10.1098/rstb.2012.0005)
 43. Gilbert AL, Miles DB. 2016 Food, temperature and endurance: effects of food deprivation on the thermal sensitivity of physiological performance. *Funct. Ecol.* **30**, 1790–1799. (doi:10.1111/1365-2435.12658)
 44. Kolbe JJ, Ehrenberger JC, Moniz HA, Angilletta MJ. 2013 Physiological variation among invasive populations of the brown anole (*Anolis sagrei*). *Physiol. Biochem. Zool.* **87**, 92–104. (doi:10.1086/672157)
 45. Gunderson AR, Stillman JH. 2015 Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B* **282**, 20150401. (doi:10.1098/rspb.2015.0401)
 46. Llewellyn J, Macdonald S, Hatcher A, Moritz C, Phillips BL. 2017 Thermoregulatory behaviour explains countergradient variation in the upper thermal limit of a rainforest skink. *Oikos* **126**, 748–757. (doi:10.1111/oik.03933)