Plasticity of Gene Expression and Thermal Tolerance: Implications for Climate Change Vulnerability in a Tropical Forest Lizard

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ABSTRACT

Tropical ectotherms are thought to be especially vulnerable to climate change because they have evolved in temporally stable thermal environments and therefore have decreased tolerance for thermal variability. Thus, they are expected to have narrow thermal tolerance ranges, live close to their upper thermal tolerance limits, and have decreased thermal acclimation capacity. Although models often predict that tropical forest ectotherms are especially vulnerable to rapid environmental shifts, these models rarely include the potential for plasticity of relevant traits. We measured phenotypic plasticity of thermal tolerance and thermal preference as well as multitissue transcriptome plasticity in response to warmer temperatures in a species that previous work has suggested is highly vulnerable to climate warming, the Panamanian slender anole lizard (*Anolis apletophallus*). We found that many genes, including heat shock proteins, were differentially expressed across tissues in response to short-term warming. Under long-term warming, the voluntary thermal maxima of lizards also increased, although thermal preference exhibited only limited plasticity. Using these data, we modeled changes in the activity time of slender anoles through the end of the century under climate change and found that plasticity should delay declines in activity time by at least two decades. Our results suggest that slender anoles, and possibly other tropical ectotherms, can alter the expression of genes and phenotypes when responding to shifting environmental temperatures and that plasticity should be considered when predicting the future of organisms under a changing climate.

Keywords: Anolis, gene expression, climate change, phenotypic plasticity, thermoregulation, RNAseq.

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Introduction

Environments on earth exist on a continuum of climatic stability that ranges from extremely stable deep-sea and cave ecosystems to volatile seasonal ecosystems of temperate latitudes (Stevens 1989, 1992; Gaston and Chown 1999). Climate variability poses a challenge to organisms because they can experience a range of abiotic and biotic conditions that test their limits of tolerance and performance. Climate varies not only across space (Sears et al. 2011; Cox et al. 2018; Fey et al. 2019) but also throughout the history of the earth (Raup and Sepkoski 1982; Markle et al. 2017). Humans have added to this temporal variability through the burning of fossil fuels, which has caused a rise in the mean and variance of environmental temperature since at least the Industrial Revolution (IPCC 2014).

While organisms may ultimately avoid climate change–driven extinction through cross-generational demographic processes like evolutionary adaptation (Salamin et al. 2010;

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Cahill et al. 2013; Logan et al. 2014), the first responses of individuals to a changing climate will occur within their lifetimes because of phenotypic plasticity. Phenotypic plasticity occurs when behavior, physiology, or morphology shifts in response to changes in the local environment (West-Eberhard 1989), and it is often underlaid by shifts in gene expression (Kelly et al. 2011; Palumbi et al. 2014). Recent research has focused on phenotypic plasticity because this process might be particularly effective at buffering organisms against short-term climate variability and may function as an important in situ adaptive response for many species (Parmesan et al. 1999; Stillman 2002; Charmantier et al. 2008; Huey and Tewksbury 2009; Breckels and Neff 2013; Gunderson and Stillman 2015; Sørensen et al. 2016; Torda et al. 2017; van Baaren and Candolin 2018; Gangloff et al. 2019; Gárate-Escamilla et al. 2019). It is important to note that several studies have suggested that plasticity alone might not adequately buffer species against climate change (Duputié et al. 2015; Oostra et al. 2018; Kellermann et al. 2020). Regardless, the plastic potential of thermal traits for most species is unknown, and models that predict the impact of climate change on populations or communities rarely account for phenotypic plasticity.

Physiological tolerances of species should be adapted to the range of environmental conditions that they experience, which should coarsely correspond to broad geographical predictors such as elevation and latitude (Janzen 1967; Shah et al. 2017, 2020). In particular, tropical species should have a narrower range of environmental tolerances, and their thermal reaction norms (the extent to which thermal performance shifts plastically with environmental temperature) should be flatter or less responsive (Stevens 1989). The latter prediction is because stable tropical temperatures should favor individuals that do not waste energy maintaining the cellular machinery needed for plastic responses when they experience only minimal environmental variation. Understanding the ways in which tropical organisms experience climate variability and their potential for plasticity is of increasing importance because their environments, which historically have been climatically stable, are now rapidly shifting as a result of anthropogenic activity.

Much of the research on temperature-induced phenotypic plasticity in tropical organisms has focused on marine environments. This research has revealed that while there is some scope for phenotypic plasticity in response to changing temperature (Grottoli et al. 2014; Seebacher et al. 2015; Kenkel and Matz 2016), plasticity is not always sufficient to withstand the negative impacts of high and variable temperatures predicted under climate change (Donelson et al. 2011; Sawall et al. 2015; Seebacher et al. 2015). However, less is known about plasticity in tropical ectotherms found in terrestrial environments, particularly those in tropical forests. Some previous research with tropical ectotherms in terrestrial environments has found that they have relatively limited plasticity to adequately buffer against a rapidly changing climate (Gunderson and Stillman 2015; Gunderson et al. 2017; Rohr et al. 2018; Montejo-Kovacevich et al. 2020; Sun et al. 2022; Turriago et al. 2022), although some studies have discovered the capacity for substantial plasticity in thermal tolerance (Llewelyn et al. 2018; Noer et al. 2022). Understanding the potential for and quantifying the magnitude of phenotypic plasticity in tropical forest ectotherms is crucial because the spatially homogeneous thermal structure of forest environments often precludes the possibility of behavioral thermoregulation. Thus, plastic shifts in thermal tolerance may be the major avenue of shorter-term in situ compensation available to tropical forest species, and in the absence of plasticity, extinction may be likely. In line with this prediction, tropical forest ectotherms have declined in abundance in recent years, with some populations having been extirpated or driven extinct as a result of habitat destruction, fragmentation, and modification by climate change (Lister and Garcia 2018; Raven and Wagner 2021; Wagner et al. 2021).

Among tropical forest ectotherms, a number of studies have suggested that tropical forest lizards are particularly vulnerable (Whitfield et al. 2007; Huey 2009; Sinervo et al. 2010). Tropical lizards often live under the shade of closed-canopy forests and have limited opportunity for behavioral thermoregulation, enhancing the potential importance of acclimation or genetic adaptation as avenues of escape from extinction (Logan et al. 2018). However, studies that predict that tropical forest lizards are particularly vulnerable to climate change have been unable to incorporate the potential for plasticity of relevant traits, given the scarcity of information. Integrating the role of plastic responses in models of the responses of tropical lizards to climate warming, from shifts in gene expression to changes in important phenotypes, may alter or reduce the predicted vulnerability of this group (Logan and Cox 2020).

We studied gene expression, phenotypic plasticity of thermal tolerance and thermal preference, and energy storage in response to temperature change in a tropical forest lizard, the Panamanian slender anole (*Anolis apletophallus*; hereafter, "slender anole"), which has been declining in abundance under contemporary climate change (Stapley et al. 2015). We combined a detailed field study of the thermal ecology of this species with (1) transcriptomic study of gene expression in response to short-term (2-h) warming and cooling, (2) assays of behavioral and physiological plasticity in response to a long-term (1-mo) greenhouse experiment, and (3) assessment of the impacts of long-term warming on lizard energetics. We then combined our field data with estimates of phenotypic plasticity of thermal tolerance to model the role of plasticity in shifting activity time as a function of climate change. Although we cannot directly assess whether the plastic responses we observed were adaptive because we did not track survival or reproductive success of individual lizards, we made a priori predictions about the kinds of plastic responses we would expect to see if these responses evolved by natural selection. For example, we predicted that an adaptive transcriptomic response to changing temperatures would be asymmetrical (greater shifts in gene expression in response to warm temperatures than to cool temperatures) because of the much greater danger to protein structure and function posed by warmer temperatures and the extreme rarity of cold conditions in the lowland tropics. We also predicted that gene expression responses would involve gene networks that respond to thermally induced damage (e.g., chaperone proteins, membrane proteins, and protein refolding). Similarly, we predicted that exposure to stressfully warm temperatures over several weeks in a greenhouse would result in plastic increases in thermal preference and tolerance and reduced energy stores as a result of

heightened basal metabolic rates. Finally, we predicted that the inclusion of phenotypic plasticity of thermal tolerance in our model of activity time would result in a less pessimistic projection for slender anole population viability under climate warming.

Methods

Study System

The slender anole is a small (<2.5-g) diurnal, semiarboreal predator that is ubiquitous throughout the lowland tropical rainforests of Panama. Slender anoles are an ideal model organism to test hypotheses about gene expression and plasticity in tropical ectotherms because they live in lowland closed-canopy forests where they are thermoconformers (i.e., slender anoles do not behaviorally thermoregulate; Logan et al. 2020), are short lived for a vertebrate (>95% annual mortality; Andrews and Nichols 1990), and are easy to maintain in captivity (Stapley et al. 2011, 2015; J. Stapley, unpublished manuscript).

Field-Active Body Temperature and Environmental Temperature

In July 2019, we captured 284 slender anoles by hand or catch pole from Soberanía National Park, Panama, and measured field-active body temperatures. During the same season, we recorded environmental temperatures using 90 data loggers positioned randomly over a large portion of our field site. We then used field-active body temperature and environmental temperature data to ensure that the thermal conditions of our gene expression, physiological, and behavioral plasticity experiments were ecologically realistic. See the appendix (available online) for details on our field-active body temperature and environmental temperature methods.

Gene Expression under Short-Term Warming and Cooling

In July 2017, we captured 24 adult male lizards from the same field site described above and transported them back to the Smithsonian facility in Gamboa, Panama. After acclimation to laboratory conditions (and fasting) for 48 h at 22°C, lizards were introduced to three Percival incubators (Percival Scientific, Perry, IA) set to a warm (32°C), control (28°C), or cool (18°C) treatment ($n = 8$ per treatment). The control treatment temperature was similar to the mean field-active body temperature for slender anoles (see "Results"). The warm treatment represents the higher end of body temperatures that slender anoles experience in the wild, as 1.1% of field-active body temperatures were above 30°C. Thus, while warm, 32°C is not far outside the normal body temperature range for a typical slender anole, and they are able to function at this temperature (Neel et al. 2021). Finally, the cool treatment represents a temperature that slender anoles might experience during rare cold snaps that likely occur once every few generations. Each lizard was maintained at the treatment temperature for 2 h before euthanasia by decapitation and immediate tissue collection of whole brain, liver, and femoral muscle.

RNA was isolated from tissue using a Trizol reagent protocol, sent to the Georgia Genomics and Bioinformatics Core for preparation of complementary DNA libraries, and sequenced on the NextSeq Illumina platform (Illumina, San Diego). Sequences were mapped to the *Anolis carolinensis* reference genome because it was the closest relative with a published genome (Alföldi et al. 2011).

We used edgeR (Robinson et al. 2010; McCarthy et al. 2012) to identify differentially expressed genes (DEGs) by conducting a pairwise analysis between the control treatment and either the warm treatment or the cool treatment for a given sample. We used a Gene Ontology (GO) analysis to identify biological processes in which DEGs were involved. Beyond global gene expression, we scrutinized the expression of candidate genes that we selected a priori (table A1; tables A1–A10 are available online) because they are known to participate in the cellular response to temperature stress (Jassal et al. 2019).

In addition to examining differential expression of individual genes, we also conducted whole-gene coexpression network analyses using the R package WGCNA (Langfelder and Horvath 2008, 2012) to test for gene modules that are coexpressed in response to acute shifts in temperature. We created separate networks for each tissue that included samples from each temperature treatment (table A2). To build the networks, genes were filtered for low expression, and read count data were normalized (Robinson et al. 2010; Robinson and Oshlack 2010) and then $\log_2$ transformed. Samples in each network were then clustered based on Euclidean distance (table A2). The data for each tissue were used to construct signed networks with minimum module sizes of 30 genes. We tested for differences in module eigengene values between experimental and control temperature treatments in all tissues using generalized linear models. We then performed gene functional enrichment analysis on genes in modules with significant differences in module eigengene values (Reimand et al. 2007). For a detailed description of our RNA-seq methods, see the appendix.

Phenotypic Plasticity, Growth, and Energetics under Long-Term Warming

We used a greenhouse experiment to assess the potential for phenotypic plasticity under long-term warming. We captured 40 lizards (equal sex ratio) in June 2019 and transported them back to the Smithsonian facility in Gamboa, Panama. After acclimation to laboratory conditions for 48 h, we measured standard morphological traits (mass and snout-vent length [SVL]) as well as a suite of physiological and behavioral traits. We measured voluntary thermal maxima (VT_{max}; an index of heat tolerance), critical thermal minima (CT_{min}; an index of cold tolerance), and body temperatures in a laboratory thermal gradient following Logan et al. (2020; see appendix for detailed methods). We calculated the mean, minimum, and maximum body temperatures chosen in the gradient as different aspects of thermal preference that might display plasticity.

After measuring phenotypes, we randomly assigned 10 males and 10 females to either a control greenhouse or a warm greenhouse (total of 20 lizards per greenhouse). Lizards were placed into 23 × 23 × 46-cm mesh cages (one individual per cage), which were themselves placed inside the greenhouse. In the control

greenhouse, we set the thermostat to 24°C for the first 5 d, 25°C for the next 5 d, and 26°C for the remaining 21 d, adjusting the temperature to recreate the natural forest thermal regime as closely as possible. In the warm greenhouse, we ramped the thermostat up from 24°C to 30°C over a period of 14 d and then held the thermostat constant at 30°C for the final 17 d. Here, we were mimicking the gradual onset of a heat wave. Hereafter, we refer to the treatments of the long-term experiment as either the "long-term warming" or the "control."

We monitored the temperatures experienced by lizards in the greenhouses by taking 549 surface body measurements during the study period using a Fluke infrared temperature gun. We used surface body temperature as our estimate of lizard temperature because the measurement of surface temperature does not require the handling of lizards and the stress from handling can affect experimental results (Foss et al. 2012). We verified that surface temperatures closely approximated cloacal temperatures (fig. A1; figs. A1, A2 are available online) by measuring both of these variables on a subset of lizards during the second week of the experiment ($n = 120$, $r = 0.88$).

After 4 wk in the greenhouses, we remeasured CT_{min}, VT_{max}, and thermal preference in a laboratory thermal gradient for 35 individuals (two lizards from the control treatment and three lizards from the heat treatment died during the experiment). We also calculated growth in terms of both SVL and mass. We then used residual body mass from a linear regression of body mass on SVL as an index of body condition (Logan et al. 2012). Finally, we dissected all individuals and weighed organs associated with energy storage and reproduction, including visceral fat bodies, livers, and gonads.

We analyzed growth and organ mass using linear regressions, including sex, treatment, and sex-by-treatment interactions as predictor variables. Body size covariates were included in models when appropriate. Differences between initial and final values (plasticity) for thermoregulatory and thermal tolerance traits were determined using a repeated-measures ANOVA. Before analyses, we ensured that all variables fit the assumptions of statistical tests. All statistical analyses were completed in JMP (ver. 12.0; JMP 2019).

Modeling the Impact of Thermal Tolerance Plasticity on Activity Time under Climate Warming

We modeled how phenotypic plasticity of thermal tolerance might alter potential activity time under climate warming. We used data and equations from Neel et al. (2021) to predict lizard body temperatures based upon projected environmental temperatures by the end of the century, assuming a 3°C increase by the year 2100 (IPCC 2018). We assumed a uniform increase in temperature of 0.0365°C yr^{-1} over that time period. We then projected future activity levels by assuming that lizard activity would cease at mean environmental temperatures exceeding VT_{max}. We projected activity time using values of VT_{max} for either the control treatment ($VT_{max} = 29.1$°C) or the warm treatment ($VT_{max} = 29.7$°C). Activity time was expressed as the percentage change in activity time relative to 2019, which was assigned a value of 100%.

Results

Field-Active Body Temperature and Environmental Temperature

Mean field-active body temperature of our study population was 27.8°C. Mean hourly environmental temperature was significantly correlated with field-active body temperature ($r = 0.69$, $P < 0.0001$), confirming that the slender anole is indeed a thermoconforming species (fig. 1*A*). We found that temporal variation in environmental temperature was minimal (fig. 1*B*), with a daytime (0700–1800 hours) mean of 26.0°C and a nighttime (1800–0700 hours) mean of 24.4°C (24-h mean: 25.2°C), and that 0.3% of environmental temperatures were above 30°C during the sampling period.

Gene Expression under Short-Term Warming and Cooling

We found a pronounced gene expression response to short-term temperature change across all three tissues, with many more genes differentially expressed in response to 32°C (warm treatment) than to 18°C (cool treatment). In the brain, relative to the control treatment, there were 5,587 genes differentially expressed in response to the warm treatment, but only one gene differentially expressed in response to the cool treatment (fig. 2*A*). Similarly, many more genes were differentially expressed in response to the warm treatment than to the cool treatment in the liver (85 genes differentially expressed in response to the warm treatment, 24 genes differentially expressed in response to the cool treatment; fig. 2*B*) and the muscle (nine genes differentially expressed in response to the warm treatment, zero genes differentially expressed in response to the cool temperature; fig. 2*C*).

Within genes that were differentially expressed, we found that many more genes were upregulated than downregulated in response to warm temperature. In the brain, we found that 3,168 genes upregulated and 2,419 genes downregulated, with a similar trend in the liver (65 genes upregulated, 20 genes downregulated) and the muscle (nine genes upregulated, zero genes downregulated). Similarly, the magnitude (average $\log_2$ fold change) of the transcriptomic response of all DEGs, regardless of tissue, was greater in response to the warm treatment than to the cool treatment. In the brain, the magnitude (average $\log_2$ fold change) of DEGs in response to the warm treatment was greater than the magnitude of DEGs in response to the cool treatment (fig. 2*D*), with a similar trend in liver and muscle tissue (fig. 2*E*, 2*F*). We also found that the magnitude of DEGs that were upregulated was greater than the magnitude of DEGs that were downregulated in response to the warm treatment across all three tissues (fig. 2*D*–2*F*). There was a positive correlation between the gene expression response to the warm treatment and the gene expression response to the cool treatment, indicating that the same genes that were upregulated in response to the warm treatment were some of the same genes that were upregulated in response to the cool treatment (fig. 2*G*–2*I*). However, these genes differ in their magnitude of expression between the warm and cool treatments. All three tissues exhibited DEGs from the cellular response to the heat pathway, suggesting that their upregulation was an adaptive response to warmer

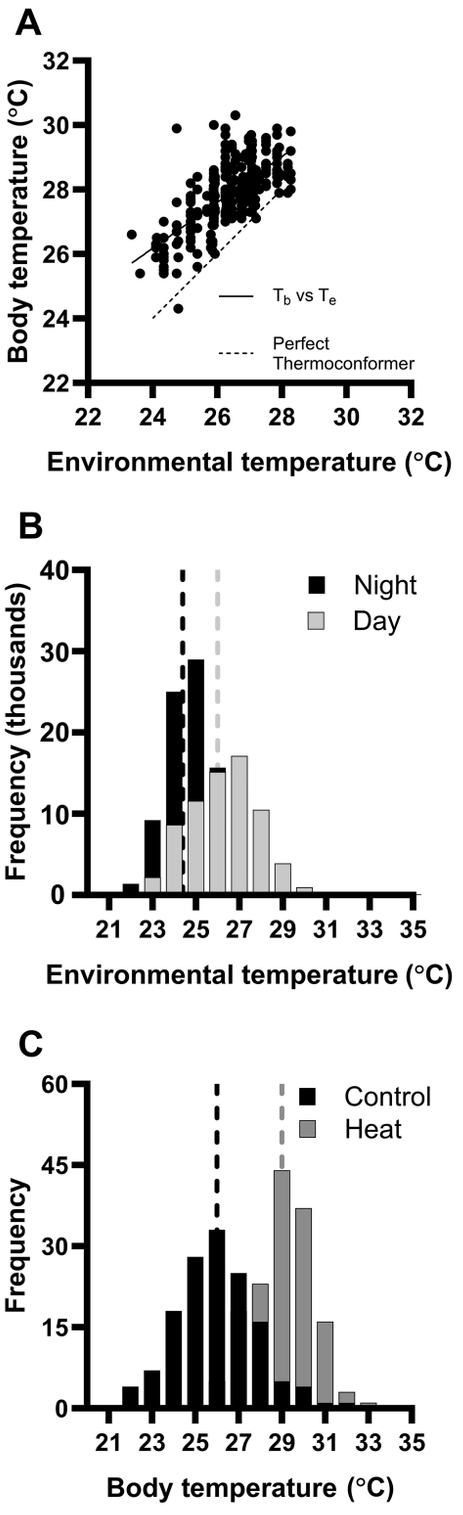

Figure 1. Field-active body temperatures (T_b's) of slender anoles, environmental temperature (T_e) distributions in their forest habitat, and (surface) T_b distributions of lizards in greenhouses. *A*, Field-active T_b's and T_e's are strongly positively correlated, a pattern that is consistent with a thermoconforming behavioral strategy and demonstrates that T_e is a good proxy for T_b in slender anoles. *B*, T_e distributions in slender anole habitat in Soberanía National Park separated by nighttime (black bars) and daytime (gray bars). The black dashed line indicates mean nighttime temperature (24.4°C), and the gray dashed line indicates mean daytime temperature (26.0°C). *C*, Frequency distributions of surface T_b's of lizards in the control treatment (black bars) and the long-term warming treatment (gray bars) during the heat wave phase of the greenhouse experiment (final 2 wk). The black dashed line indicates mean surface T_b during daytime for the control treatment (26.0°C), and the gray dashed line represents mean surface T_b during daytime for the long-term warming treatment (29.0°C). The surface T_b's of lizards in the long-term warming treatment were higher than both the daytime T_e's in the forest and the surface T_b's of lizards in the control treatment.

temperatures (table A3). The pattern of gene expression for DEGs in this pathway mirrored what was found in global gene expression for all three tissues (fig. A2).

DEGs in the liver and the muscle generally belonged to biological processes that protect proteins from degradation (table 1). Genes related to the heat shock protein (HSP) families HSP40, HSP70, and HSP90 were represented in six GO terms from the liver and the muscle. By contrast, the brain had 214 significantly enriched biological processes without enrichment of protein folding processes but that did include enrichment for protein ubiquitination and ubiquitin-dependent protein catabolic process, which could indicate an increase of protein degradation (Glickman and Ciechanover 2002).

We found that tissue-specific networks contained many different modules (table A4), some of which were associated with temperature treatments. Three modules in the brain network (blue, green, and turquoise), one module in the liver network (green), and one module in the muscle network (black) had significant differences in module expression between the high and control temperature treatments (table A5), while there were no significant differences between the low temperature treatment and the control temperature treatment in any module. These modules contained genes that were significantly enriched for many biological processes, including development, response to stress, cellular regulation, and metabolism (table A6). Of the candidate genes (i.e., identified a priori) for the response to temperature, 38 were included in tissue-specific networks and assigned to modules. Of these genes, 18 were assigned to the blue, green, and turquoise modules that had significant differences in module eigengene values between the high temperature treatment and the control temperature treatment (table A7). We also found substantial overlap between modules that were significantly associated with temperature treatments and genes that were identified as being differentially expressed in the edgeR analytical pipeline. The blue module was composed of 735 DEGs from edgeR out of a total of 992 genes. The green module was composed of 320 DEGs from edgeR out of a total of 444 genes. The turquoise module was composed of 197 DEGs from edgeR out of a total of 1,203 genes.

Body Temperature, Growth, and Body Condition under Long-Term Warming

During the final 2 wk of the greenhouse experiment (after lizards had endured the warm treatment "heat wave" for 2 wk),

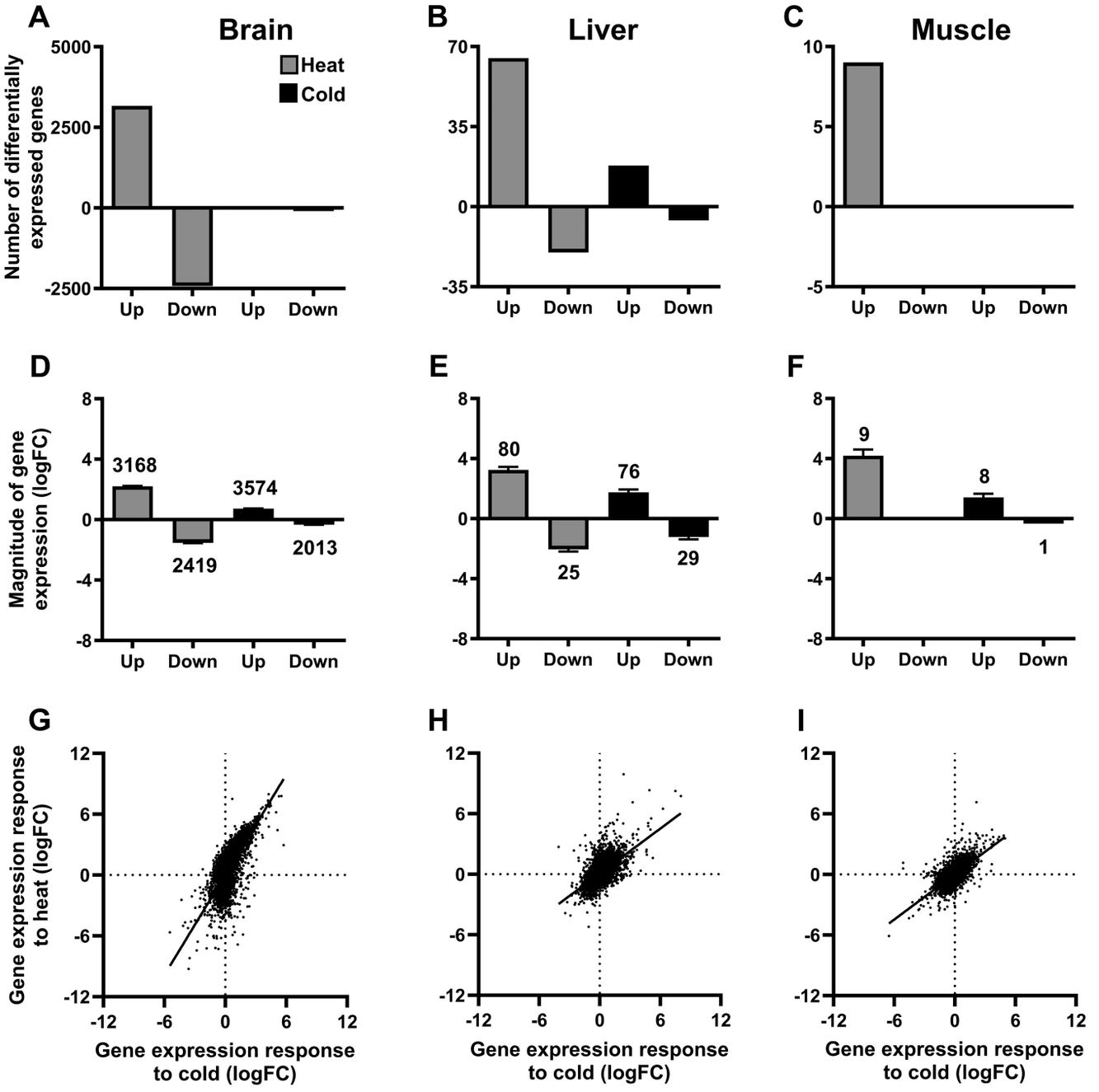

Figure 2. Transcriptomic response to short-term warming and cooling in the brain, liver, and muscle of slender anoles. *A–C*, Number of differentially expressed genes in response to heat (gray bars) and cold (black bars) compared to the control treatment (false discovery rate < 0.05) for the brain (*A*), the liver (*B*), and the muscle (*C*). Positive values are genes that were upregulated, and negative values are genes that were downregulated. All three tissues shifted gene expression in a putatively adaptive direction by differentially expressing a greater number of genes in response to heat than to cold. *D–F*, Magnitude of the transcriptomic response (average $\log_2$ fold change [logFC]) to heat is greater than that of the response to cold across all three tissues. Numbers above the bars indicate the number of differentially expressed genes in response to both treatments. Error bars represent standard error. *G–I*, Across all tissues, genes that were up- or downregulated in response to short-term warming also tended to be regulated in the same direction in response to short-term cooling and vice versa. Each point is an individual gene.

Table 1: Differently expressed genes (DEGs) from liver and muscle that represented two and nine significantly enriched Gene Ontology (GO) terms, respectively

Tissue, GO biological process	Gene ID	Gene	log_2FC	Panther family
Liver:				
Protein refolding (GO:0006457)	ENSACAT00000030524	*LOC100554364*	5.39	Hsp70
	ENSACAT00000006050	*DNAJA4*	5.10	Chaperone
	ENSACAT00000004906	*HSPA8*	2.19	Hsp70
	ENSACAT00000013542	*DNAJB2*	2.08	Chaperone
	ENSACAT00000015808	*HSPA2*	9.90	Hsp70
	ENSACAT00000011882	*HSPA4L*	2.04	Hsp70
	ENSACAT00000017313	*AHSA1*	2.03	Chaperone
	ENSACAT00000000159	*HSP90AA1*	4.80	Hsp90
Protein refolding (GO:0042026)	ENSACAT00000030524	*LOC100554364*	5.39	Hsp70
	ENSACAT00000006050	*DNAJA4*	5.10	Chaperone
	ENSACAT00000004906	*HSPA8*	2.19	Hsp70
	ENSACAT00000015808	*HSPA2*	9.90	Hsp70
Muscle:				
Negative regulation of inclusion body assembly (GO:0090084)	ENSACAT00000006050	*DNAJA4*	3.19	Chaperone
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
Regulation of inclusion body assembly (GO:0090083)	ENSACAT00000006050	*DNAJA4*	3.19	Chaperone
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
Protein refolding (GO:0042026)	ENSACAT00000030524	*LOC100554364*	3.21	Hsp70
	ENSACAT00000006050	*DNAJA4*	3.19	Chaperone
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
Response to cold (GO:0009409)	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
	ENSACAT00000000159	*HSP90AA1*	3.57	Hsp90
Chaperone cofactor-dependent protein refolding (GO:0051085)	ENSACAT00000030524	*LOC100554364*	3.21	Hsp70
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
Response to heat (GO:0009408)	ENSACAT00000006050	*DNAJA4*	3.19	Chaperone
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
	ENSACAT00000000159	*HSP90AA1*	3.57	Hsp90
Response to temperature stimulus (GO:0009266)	ENSACAT00000006050	*DNAJA4*	3.19	Chaperone
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
	ENSACAT00000000159	*HSP90AA1*	3.57	Hsp90
Protein folding (GO:0006457)	ENSACAT00000030524	*LOC100554364*	3.21	Hsp70
	ENSACAT00000006050	*DNAJA4*	3.19	Chaperone
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
	ENSACAT00000000159	*HSP90AA1*	3.57	Hsp90
Response to abiotic stimulus (GO:0009628)	ENSACAT00000006050	*DNAJA4*	3.19	Chaperone
	ENSACAT00000007045	*JUN*	3.87	Basic leucine zipper transcription factor
	ENSACAT00000015808	*HSPA2*	7.14	Hsp70
	ENSACAT00000000159	*HSP90AA1*	3.57	Hsp90

Note. For each tissue in the warm treatment, we compiled all DEGs into a list and then input each list into the Gene Ontology Resource (Ashburner et al. 2000; Gene Ontology Consortium 2018) that uses the analysis tool from the Panther Classification System (Mi et al. 2018) to identify the biological processes for which DEGs were most involved. Significantly enriched processes were identified as those that had an false discovery rate <0.05. Biological processes typical of response to heat, such as protein refolding (GO:0006457 and GO:0042026), chaperone cofactor-dependent protein refolding (GO:0051085), response to heat (GO:0009408), response to temperature stimulus (GO:0009266), protein folding (GO:0006457), and response to abiotic stimulus (GO:0009628), were significantly enriched when we analyzed 85 DEGs from the liver and nine DEGs from the muscle. Heat shock proteins (HSPs) represented some of the most highly expressed transcripts. log_2FC = average log_2 fold change.

lizards exposed to long-term warming experienced a daytime (0700–1800 hours) mean surface body temperature of 29.0°C ($n = 159$, 27.7% ≥ 30°C) and a nighttime (1800–0700 hours) mean surface body temperature of 26.1°C ($n = 38$). Lizards in the control treatment were substantially cooler, with a daytime mean surface body temperature of 26.0°C ($n = 149$, 2.0% ≥ 30°C) and a nighttime mean surface body temperature of 25.4°C ($n = 36$). Control lizards remained at temperatures close to the mean environmental temperature of their field site (25.2°C; fig. 1*B*, 1*C*).

We found that several measures of growth and energy storage differed between lizards in the long-term warming and control treatments. Lizards in the long-term warming treatment grew more rapidly in SVL than lizards in the control treatment (treatment, $F_{3, 30} = 5.2806$, $P = 0.0287$; sex, $F_{3, 30} = 0.2425$, $P = 0.6260$; treatment-by-sex interaction, $F_{3, 30} = 0.4311$, $P = 0.5165$), whereas growth in mass did not differ between treatments (treatment, $F_{3, 30} = 1.2959$, $P = 0.2640$; sex, $F_{3, 30} = 0.9495$, $P = 0.3376$; treatment-by-sex interaction, $F_{3, 30} = 0.1016$, $P = 0.7521$). Body condition did not vary between treatments, although females had a higher body condition than males across both treatments (treatment, $F_{3, 30} = 0.2395$, $P = 0.6281$; sex, $F_{3, 30} = 6.0863$, $P = 0.0196$; treatment-by-sex interaction, $F_{3, 30} = 0.0016$, $P = 0.9688$). We found that visceral fat body mass differed between treatments in a sex-dependent fashion (treatment, $F_{4, 30} = 0.6679$, $P = 0.4168$; sex, $F_{4, 30} = 3.7484$, $P = 0.0623$; treatment-by-sex interaction, $F_{4, 30} = 5.2999$, $P = 0.0284$; body mass, $F_{4, 30} = 5.1794$, $P = 0.0302$). At the end of the experiment, control females had larger fat bodies than warm-treatment females, while the opposite was true for males (fig. 3). Liver mass did not vary between treatments, although females had larger livers than males across both treatments (treatment, $F_{4, 29} = 0.0074$, $P = 0.9321$; sex, $F_{4, 29} = 4.8079$, $P = 0.0365$; treatment-by-sex interaction, $F_{4, 29} = 0.1380$, $P = 0.7130$; body mass, $F_{4, 29} = 10.0177$, $P = 0.0036$). The size of the gonads did not differ between treatments for either females (treatment, $F_{2, 13} = 2.5568$, $P = 0.1338$; body mass, $F_{2, 13} = 5.2283$, $P = 0.0396$) or males (treatment, $F_{2, 15} = 0.7478$, $P = 0.4008$; body mass, $F_{2, 15} = 5.4034$, $P = 0.0345$).

Plasticity of Thermal Tolerance under Long-Term Warming

We found stronger evidence of plasticity in upper thermal tolerance than in lower thermal tolerance in slender anoles. When lizards from the warm or cool greenhouses were analyzed separately, there was a significant increase in VT_{max} following exposure to long-term warming ($F_{1, 17} = 10.23$, $P = 0.0053$) but not in the control treatment ($F_{1, 18} = 1.4568$, $P = 0.2431$). Average CT_{min} decreased slightly in both treatments, but this change was not significant (control, $F_{1, 18} = 1.408$, $P = 0.2513$; long-term warming, $F_{1, 17} = 3.9265$, $P = 0.639$; fig. 4*A*). We also analyzed our results by pooling all individuals in both treatments. In this expanded repeated-measures model that included treatment (cool or warm greenhouse), time (pre- or postexposure), sex, body mass, and their respective interactions, we found no effect of mass on VT_{max} or CT_{min}, although CT_{min} differed by sex (table A8). We did not find a significant interaction between the repeated factor (time) and the temperature treatment in the expanded model (table A8). See table A9 for VT_{max} and CT_{min} means and standard errors for males, females, and treatments.

Plasticity of Thermal Preference under Long-Term Warming

We detected little evidence for plasticity of thermal preference in response to long-term warming in the slender anole. We did

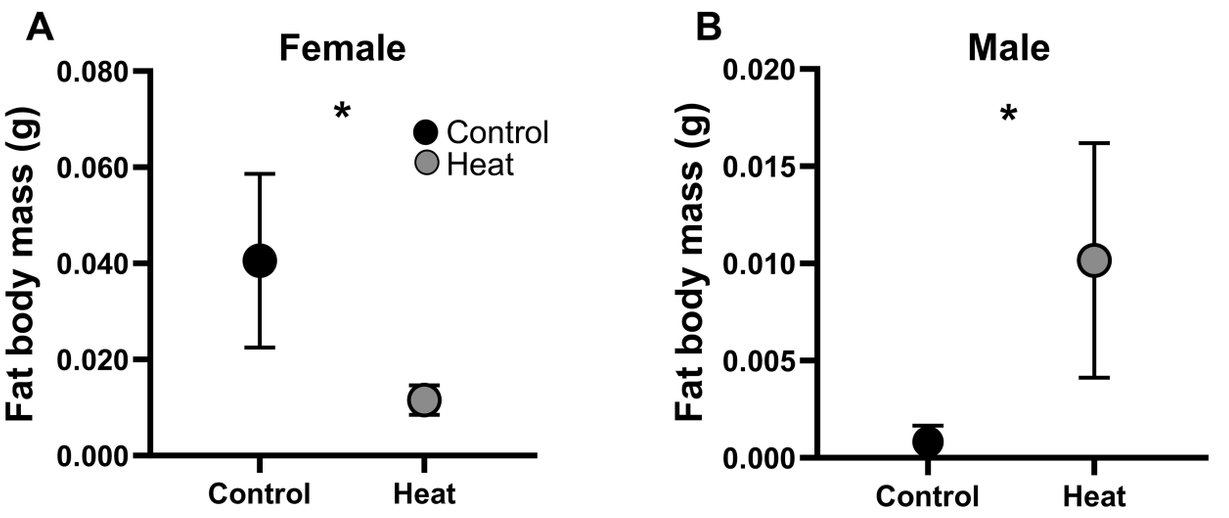

Figure 3. Energy stores of male and female slender anoles after 1 mo in control conditions (black points) or long-term warming conditions (gray points) in greenhouses. Error bars represent standard error. *A*, Control females had significantly larger visceral fat bodies than warm-treatment females. *B*, Control males had significantly smaller visceral fat bodies than warm-treatment males. These results suggest a sex-specific impact of long-term warming on energy storage. Asterisks denote significant differences.

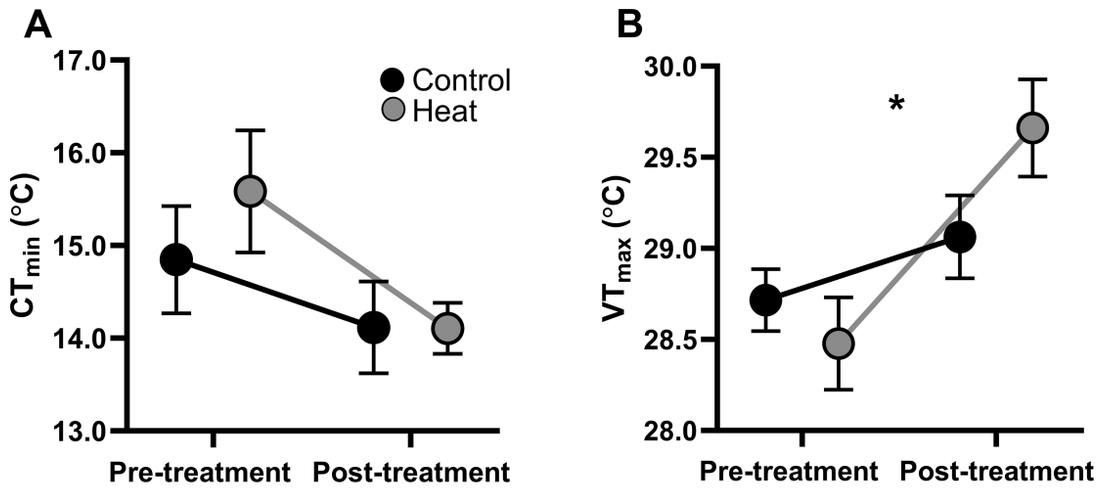

Figure 4. Critical thermal minima (CT_{min}) and voluntary thermal maxima (VT_{max}) of slender anoles before and after 1 mo of exposure to a control treatment (black) or a long-term warming treatment (gray). Error bars represent standard error. *A*, Average CT_{min} decreased slightly but did not change significantly after exposure to either treatment. *B*, Average VT_{max} did not change in control lizards but increased significantly in warm-treatment lizards. The asterisk denotes significant differences.

not find a significant change in the mean (long-term warming, $F_{1,17} = 1.2869, P = 0.2724$; control, $F_{1,16} = 4.4933, P = 0.05$) or maximum (long-term warming, $F_{1,17} = 0.0560$, $P = 0.8158$; control, $F_{1,16} = 0.1678$, $P = 0.6875$) body temperature chosen in a thermal gradient after exposure to either the control treatment or the long-term warming treatment. By contrast, the minimum temperature chosen in a thermal gradient decreased significantly in the control treatment ($F_{1,16} = 13.9098$, $P = 0.0018$) but did not change following exposure to long-term warming ($F_{1,17} = 0.5503$, $P = 0.4683$; fig. 5*A*). Again, we analyzed these data using an additional expanded model that pooled all individuals across both treatments. In this expanded repeated-measures model that included treatment (cool or warm greenhouse), time (pre- or post-exposure), sex, body mass, and their respective interactions, we found no effect of any of these factors on the mean, maximum, or minimum temperature chosen in a thermal gradient after exposure to either the control treatment or the long-term warming treatment (table A10).

Impact of Phenotypic Plasticity on Activity Time under Climate Warming

Incorporating the phenotypic plasticity of thermal tolerance (VT_{max}) into projections of activity time substantially lessened

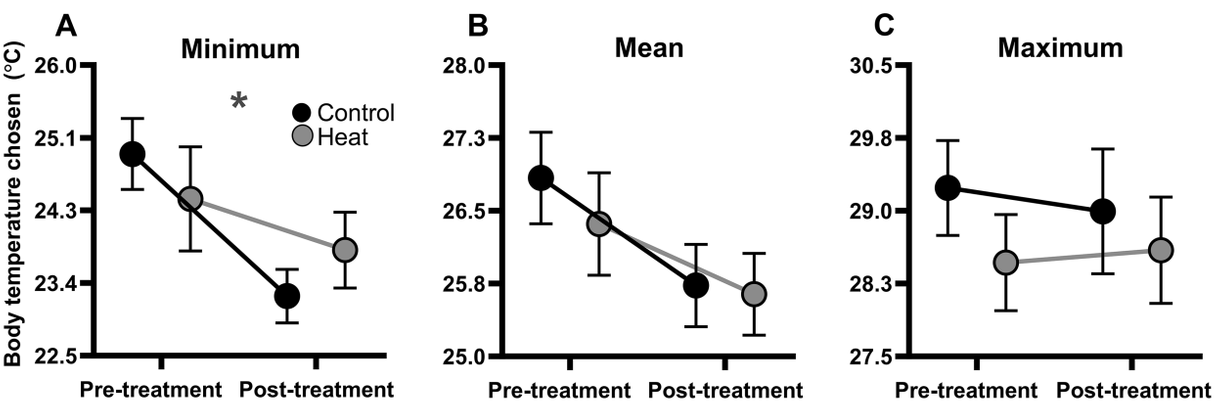

Figure 5. Body temperatures chosen in a laboratory thermal gradient before and after 1 mo of exposure to a control treatment (black) or a long-term warming treatment (gray) in the slender anole. Error bars represent standard error. *A*, Minimum body temperature chosen in a thermal gradient decreased significantly in response to the control treatment but not in response to long-term warming. *B*, *C*, Neither mean (*B*) nor maximum (*C*) body temperature chosen in a thermal gradient differed in response to either the control treatment or the long-term warming treatment.

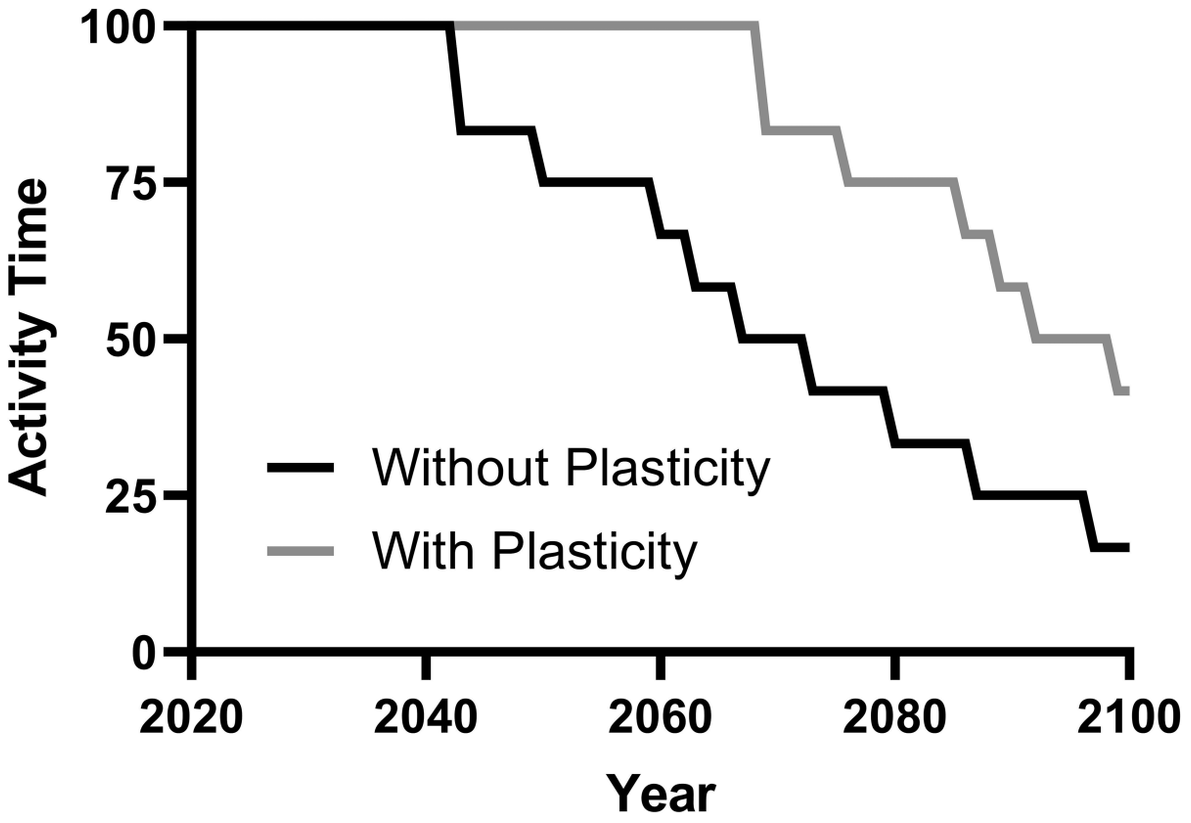

Figure 6. Projections of the potential activity time of our focal population of slender anoles made both with and without a capacity to alter thermal tolerance (voluntary thermal maxima [VT_{max}]) using phenotypic plasticity. Our model assumes that lizards become inactive when mean environmental temperature exceeds the mean VT_{max} of the population. We assumed that mean daytime temperature would increase uniformly by 3°C by the end of the century (0.0365°C yr^{-1} until 2100). When phenotypic plasticity is incorporated into projections of activity time, initial reductions in activity time are delayed by 21 yr, and by the end of the century, total activity time is reduced to 25% of present-day activity compared to just 8.3% when plasticity is not included.

reductions in activity time caused by climate warming. While the model that did not incorporate plasticity of thermal tolerance projected that activity time would be reduced by 2039, the model that included plasticity projected that activity time would not be reduced until 2060 (fig. 6). In addition, while activity time was predicted to be only 8.3% of present-day activity by the year 2100 in models that did not incorporate plasticity, it was predicted to be 25% of present-day activity by the year 2100 in the model that included plasticity (fig. 6).

Discussion

Previous studies have implied that terrestrial ectotherms inhabiting the tropics might have restricted plasticity of thermal physiology (Janzen 1967; Chown et al. 2004; Ghalambor et al. 2006; Bozinovic et al. 2011) and that the capacity for plasticity to buffer terrestrial ectotherms from climate change may be limited (Gunderson and Stillman 2015). Nevertheless, we found evidence of transcriptomic and phenotypic plasticity of thermal tolerance in a putatively adaptive direction in response to changing temperatures in a tropical ectotherm, the slender anole lizard. While tropical forest lizards are predicted to be negatively impacted by climate change (Huey 2009; Sinervo et al. 2010), our results suggest that at least some species might be capable of using within-generation adaptive processes like gene expression and phenotypic plasticity to respond to changing conditions. For example, in our short-term experiment, three vital organs shifted gene expression during exposure to relatively mild increases in temperature, and these shifts included canonical HSPs, implying that changes in transcriptome regulation occurred as a direct functional response to thermal stress. During the long-term (greenhouse) experiment, slender anoles exposed to month-long warming ended with greater VT_{max} than those exposed to control (average field) conditions (fig. 4*B*). These results suggest that thermal tolerance plasticity in slender anoles would extend normal activity time by at least an additional 21 yr under climate change (fig. 6). Taken together, our results suggest that both short-term and long-term temperature shifts can instigate a plastic response of gene expression and thermal tolerance that could be important in the response to climate change.

Slender anoles differentially expressed more genes and displayed a greater magnitude of expression of those genes in response to warm conditions than to cool conditions (fig. 2*A*–2*F*). The greater response of gene expression to warm conditions might reflect the asymmetrical fitness costs of warm versus cool temperatures (Martin and Huey 2008). While extreme or longer-term cooling can exert selection, impede feeding and reproduction, and generate an increased risk of disease in squamate reptiles (Campbell-Staton et al. 2016, 2017; Lorch et al. 2016; Vicente Liz et al. 2019), short-term cooling often has only minor impacts on fitness, only transiently reducing physiological performance with little risk of permanent damage to the organism (Hochachka and Somero 2002). By contrast, even short-term exposure to increased temperature can disrupt cell membrane structure, alter enzyme activity, and cause protein denaturation, all of which can have dire consequences for fitness (Michaelis and Menten 1913; Johnson and Goody 2011). Nevertheless, given that we occasionally observed lizards in nature with body temperatures above 30°C (even during a relatively short sampling period), we were surprised to find such pronounced gene expression responses to only 2 h of exposure to a 32°C treatment. This suggests that wild slender anoles may alter gene expression in response to relatively mild week-to-week (or even day-to-day) shifts in thermal environments within the forest.

Gene expression responses to short-term temperature change were also somewhat tissue specific. For example, the brain exhibited the strongest gene expression response to increased temperature, with many more genes upregulated in the brain than in the liver or the muscle. Similar to our study, Akashi et al. (2016) found a higher number of DEGs in the brain by exposing three Cuban anole species to increased temperatures. This dramatic gene expression response to increased temperature in the brain could reflect a greater thermal sensitivity of this organ, the high fitness cost of damaging this organ, or a combination of both of these factors. GO analysis identified several protein-folding-related biological processes that were significantly enriched in the liver and the muscle (table 1). In addition, we identified DEGs of three families of HSPs (HSP70, HSP40, and HSP90) in the brain, the liver, and the muscle (table A3). HSPs are "molecular chaperones" that serve many biological functions but are most well known for their role in stabilizing the structure of proteins as they begin to denature under heat shock (Ritossa 1962; Richter et al. 2010), and their patterns of expression are frequently used to infer how organisms might respond to climate change (Tomanek 2010; González et al. 2016). Our results indicate that slender anoles can alter gene expression to mitigate the fitness costs of temperature shock and that they do this in a tissue-specific fashion, over short timescales, and in response to only moderate increases in temperature.

Although we observed substantial changes in gene expression when slender anoles were exposed to thermal stress, it is important to note that transcriptomic plasticity might not always result in phenotypic plasticity, as expression of some genes could increase or decrease plasticity at the phenotypic level. Although a framework for interpreting gene expression in the context of stress tolerance has been proposed (Rivera et al. 2021), interpreting the direct impact of gene expression on thermal tolerance range and organismal phenotype is difficult. Given the differences in timescales, we cannot extend our transcriptome plasticity results from our short-term warming or cooling experiment to our long-term warming experiment biological pathways already discussed. However, the gene expression response we measured does have an interesting parallel to acclimation responses recorded in many ectotherms. Often, acclimation to high temperatures is followed by a shift in critical thermal maxima (CT_{max}) and CT_{min}, such that there is a correlated response at the edges of the thermal tolerance range (Lowe and Vance 1955; Wilhoft and Anderson 1960; Yang et al. 2008; Li et al. 2009; Wang et al. 2013). We found that the magnitude of the gene expression response to short-term warming or cooling was also highly correlated (fig. 2*G*–2*I*), indicating that responses to both cool and warm temperatures are similar in direction of regulation, if not magnitude. Thus, the plastic responses to warm and cool temperatures are mediated by the same transcriptomic pathways, and selection for an increased gene expression response to warm conditions will likely result in a correlated response to cool conditions, although debate about this type of correlated response has been raised by Mallard et al. (2018) and Ghalambor et al. (2018). Nevertheless, further research is needed to establish the mechanistic links between transcriptomic and phenotypic plasticity of thermal tolerance because it will be crucial for understanding the role of gene expression in organismal responses to shifting temperatures.

We found that the voluntary thermal maximum increases when slender anoles are exposed to long-term warming (fig. 4*A*). However, we found little evidence of plasticity in thermal preference. Previous work has predicted that plasticity of thermal traits should decrease with latitude (a proxy for climate variability) and be minimal in the tropics (Gaston 2003; Bozinovic et al. 2011). Understanding the generality of these patterns is crucial, as it has led to pessimistic predictions about the impact of climate change on tropical ectotherms generally and lizards specifically (Deutsch et al. 2008; Tewksbury et al. 2008; Huey 2009). Previous work has also predicted that the plasticity of thermal limits will not effectively buffer organisms from changes in temperature as climate change progresses (Gunderson and Stillman 2015; Sun et al. 2022). It is worth noting that we found no change in the critical thermal minimum; however, this may differ for mid- and high-altitude species that are exposed to more cold variation than their lowland congeners (Huang et al. 2007). Taken together, our results suggest that at least one species of tropical forest lizard can adjust its heat tolerance in order to respond to long-term increases in temperature.

When we included thermal tolerance (VT_{max}) plasticity into projections of activity time, this resulted in more optimistic predictions for the impact of climate change on slender anoles (fig. 6). Activity time in our model depended entirely on the relationship between environmental temperature and VT_{max}; however, activity time can be influenced by a range of factors, including precipitation (Reynolds 1982), predators (Ferguson et al. 1988), prey (Smith et al. 2019), and competitors (Farris et al. 2015). Climate change might alter activity time in a way that is not captured by our simple model. Regardless, diel variation in environmental temperature is considered a major driver of activity time in many lizard species (Grant and Dunham 1988; Grant 1990; Sinervo et al. 2010;

Logan et al. 2015; Gunderson and Leal 2016); thus, our model may capture an important component of the relationship between climate change and slender anole activity patterns. Our results underscore the importance of including the role of phenotypic plasticity in models that predict how tropical forest lizards respond to climate change.

Warming altered the energetics of slender anoles in a sex-specific fashion. Long-term warming was associated with an increase in visceral fat body mass in males, whereas females experienced a decrease in fat body mass while maintaining ovarian mass. While warming stimulated increased growth in both sexes (despite a likely increase in maintenance metabolism with warmer temperatures; Logan 2019), males allocated energy toward growth and storage while females shunted energy toward growth and reproduction. In our experiment, slender anoles were given food ad lib., which might have obviated the energetic trade-offs between growth, storage, and reproduction. Thus, these results may have limited ecological relevance, as tropical ectotherms in nature experience seasonal limitations in food (Christian et al. 1999). Regardless, previous research has found that reproduction results in lower energy stores and confers a substantial fitness cost in female anoles (Cox et al. 2010; Cox and Calsbeek 2010; Reedy et al. 2016). Accordingly, our work suggests that food limitation might interact with increased temperatures (and maintenance metabolism) to alter growth, reproduction, and energy storage in a sex-specific fashion.

As has been suggested recently (e.g., Gunderson and Stillman 2015), our data from the slender anole support the assertion that tropical ectotherms may have greater thermal-acclimation capacity than historically acknowledged. This may follow from several features of tropical terrestrial environments. First, although these species live in "predictable" thermal environments from a broad temporal and spatial perspective (i.e., over geological time and relative to temperate latitudes), tropical environments may be relatively unpredictable on the finer spatial and temporal scales experienced by actual organisms (Logan et al. 2013; Potter et al. 2013). It is these fine-grained thermal environments that are relevant to acute reversible forms of plasticity. Second, having evolved in these geologically stable thermal environments, tropical ectotherms have narrow basal thermal tolerance ranges (Ghalambor et al. 2006). As such, even relatively minor shifts in temperature could substantially impact their fitness, such that short-term physiological plasticity might be a critical compensatory mechanism. Indeed, a recent study by Stroud et al. (2020) revealed that an entire assemblage of subtropical lizards shifted their physiology in response to rapid changes in temperature in a manner consistent with either plasticity or selection. In the case of the slender anole, even relatively mild exposure to warm conditions (2 h at 32°C, which is close to the upper body temperatures they experience on a week-to-week basis in nature) was enough to generate a detectable gene expression response (including the upregulation of HSPs). Given the narrow thermal tolerance ranges of tropical ectotherms, it seems possible that short-term reversible plasticity could be even more common in low-latitude species than previously thought. Nevertheless, to our knowledge, this prediction has not been empirically tested. Future work should test whether latitudinal patterns of short-term reversible plasticity differ from those of fixed developmental plasticity. In general, our results suggest that some tropical forest ectotherms may have the ability to at least partially mitigate the detrimental effects of rapid environmental change with plastic shifts in gene expression and phenotypes.

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