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Plasticity and the Impact of Increasing Temperature on a Tropical Ectotherm

Adam A. Rosso

PLASTICITY AND THE IMPAPCT OF INCREASING TEMPERATURE

ON A TROPICAL ECTOTHERM

by

ADAM A. ROSSO

(Under the direction of Christian L. Cox)

ABSTRACT

Organisms may respond to climate change through behavior, genetic adaptation, and/or phenotypic plasticity. Tropical ectotherms are thought to be especially vulnerable to climate change because most have a narrow range of thermal tolerance while living close to their upper thermal tolerance limits. Additionally, many tropical species live in closed-canopy forests, which provide homogenous thermal landscapes that prevent behavioral compensation for stressfully warm temperatures. Finally, tropical ectotherms are thought to have decreased capacity for phenotypic plasticity because they have evolved in thermally stable environments. We tested gene expression patterns and phenotypic plasticity in the Panamanian slender anole by a) measuring changes in gene expression in response to, short-term temperature change (two hours) and b) using a mesocosm experiment to measure phenotypic plasticity in response to longer-term thermal stress (one month). In response to short-term exposure, we found the brain, liver, and muscle differentially expressed genes (DEGs) that coded for heat shock proteins. Interestingly, all three tissues displayed a greater gene expression response to warm conditions relative to cool conditions. During longer-term exposure (mesocosm experiment), we found that lizards exposed to heat treatment had increased VT_{max} and had limited plasticity of thermoregulatory behavior. Our results provide evidence that tropical forest lizards can use gene expression and phenotypic plasticity to respond to shifting environmental temperatures, despite having evolved under

thermally stable conditions. This work suggests that genomic regions that regulate pathways of heat shock response will likely be under selection in response to global climate change. Gene expression and phenotypic plasticity are processes that should be considered when predicting the future of tropical ectotherms under a changing climate.

INDEX WORDS: Anolis, Gene expression, Phenotypic plasticity, Plasticity, Thermal adaptation, Thermoregulation, Tropical ectotherms, RNA seq

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DEDICATION

To my parents, siblings, tíos, tías, cousins and nephews who have supported me. Your love for life and nature has helped me grow.

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CHAPTER 1

INTRODUCTION

Purpose of the study

During the past century, earth's surface temperature has risen at an alarming rate and is expected to continue to increase (IPCC 2001). This change in climate is predicted to modify ecosystems worldwide with the most concentrated changes occurring in tropical and subtropical regions (Williams, Jackson & Kutzbach 2007). Although the tropics have been climatically stable since the Pliocene (Ruddiman 2001; Herbert et al. 2010), they are expected to experience decreased precipitation (Kutzbach, Williams & Vavrus 2005) and increased temperatures (Lyra et al. 2017). Hence, tropical organisms are threatened by climate change because they have adapted to stable environments which has resulted in narrow distributions and narrow thermal tolerances for many tropical species (Ghalambor et al. 2006; Menzel et al. 2006). These characteristics of tropical organisms suggest that a small shift in climate may negatively affect performance. Yet, understanding how organisms will persist in response to change in climate in the near future is not quite clear.

Organisms can respond to climate change in four ways: genetic adaptation (Thompson et al. 2013), phenotypic plasticity (Nicotra et al. 2010), migration (Parmesan et al. 1999) and extinction (Cahill et al. 2013). Studies have focused on genetic adaptation (Hancock et al. 2011; Hoffmann & Sgrò 2011; Franks & Hoffmann 2012; Logan et al. 2018; Logan et al. 2020) but there has been increasing attention paid to other processes such as behavior (van Baaren & Candolin 2018) and phenotypic plasticity (Breckels & Neff 2013; Gunderson & Stillman 2015; Sørensen, Kristensen & Overgaard 2016; Torda et al. 2017; Gangloff et al. 2019; Gárate-

Escamilla et al. 2019). Research on plastic responses to climate change has focused on the plasticity of thermal traits (Seebacher, White & Franklin 2014) and the patterns of gene expression underlying these traits (Palumbi et al. 2014). However, there are conflicting perspectives on how phenotypic plasticity affects the fitness of populations (Ancel 2000; Price, Qvarnström & Irwin 2003; Ghalambor et al. 2007). Further, there are multiple hypotheses for how phenotypic plasticity interacts with genetic adaptation, such as the Baldwin effect, genetic assimilation and genetic compensation (Baldwin 1902; Waddington 1961; Grether 2005; Corl et al. 2018). Thus, it remains unclear whether gene expression and phenotypic plasticity can aid in the response to climate change (Lancaster et al. 2016; Arnold, Nicotra & Kruuk 2019).

There are several hypotheses that try to predict large scale patterns of phenotypic plasticity. The Climatic Variability Hypothesis states that thermal tolerance ranges and phenotypic plasticity should decrease toward the equator (Gutiérrez-Pesquera et al. 2016) because tropical organisms have evolved in thermally stable environments. While studies have partially supported this hypothesis by demonstrating that the basal thermal tolerance range is often reduced in tropical ectotherms, (Janzen 1967; Ghalambor et al. 2006; Deutsch et al. 2008; Tewksbury, Huey & Deutsch 2008; Huey 2009) there is conflicting evidence over whether the plasticity of these traits is also reduced (Gunderson & Stillman 2015). Other studies support the trade-off hypothesis which states organisms with the highest thermal tolerance should display lower plasticity in thermal tolerance (Gause 1942; Somero & DeVries 1967; Chown 2001; Somero 2010; Overgaard et al. 2011). This hypothesis suggests that organisms from extreme environments with the highest thermal tolerance may be the most vulnerable to climate change. Both hypotheses imply that understanding physiological plasticity will be critical for

understanding the response of tropical organisms to climate change yet predicting this response remains unknown for many groups, such as tropical ectotherms.

Over the past two decades scientists have revealed a decline in tropical lizards around the world and predict that many will go extinct due to habitat destruction, fragmentation and modification by climate change (Whitfield et al. 2007; Sinervo et al. 2010). Specifically, tropical lizards are at risk of extinction because most have narrow thermal tolerance ranges while living close to their upper thermal tolerance limits (Huey 2009; Sinervo et al. 2010). Further, many tropical lizards live under shade of closed-canopy forests and have limited opportunity for behavioral thermoregulation. It is unclear whether tropical lizards and other tropical ectotherms can keep pace with climate change, thus understanding the role of adaptive phenotypic plasticity is crucial for predicting population persistence under climate change.

We tested the gene expression patterns and phenotypic plasticity in a thermoconforming, tropical lowland forest lizard, the Panamanian slender anole (*Anolis apletophallus*), by 1) measuring changes in gene expression in response to short-term temperature change and 2) using a mesocosm experiment to measure phenotypic plasticity in response to longer-term thermal stress. Here we define phenotypic plasticity as any change in morphology, physiology, or behavioral strategy in response to a change in the environment. We define adaptive gene expression and adaptive phenotypic plasticity as a response that improves the match between phenotype and environment.

Regulation of gene expression underlies changes in phenotype (Schoffl & Panikulangara 2008; Schunter et al. 2016). Conserved families of proteins, such as heat shock protein families are upregulated in response to environmental stress (Richter, Haslbeck & Buchner 2010). Adaptive gene expression should be represented by the heat shock response because heat shock

proteins are a highly conserved group of proteins and are involved in rescuing many macromolecules from denaturing under thermal stress (Richter, Haslbeck & Buchner 2010). We predicted that short-term temperature change would increase transcription of genes associated with heat shock response. Heat shock response has been correlated with plasticity of plasticity of heat shock response (Buckley & Hofmann 2002) which indicates that lizards in longer-term thermal stress may respond by adaptive phenotypic plasticity.

Phenotypic plasticity of physiological and behavioral traits can occur in weeks to months (Denver 1997; Tollrian & Harvell 1999). Thus, investigating physiology and behavioral thermoregulation in response to increasing temperature during this time scale may reveal adaptive phenotypic plasticity. For our longer-term thermal stress experiment, we predicted lizards would exhibit adaptive phenotypic plasticity of physiology, and thermoregulatory behaviors. These data will allow us to better predict the responses of tropical ectotherms to global climate change and provide insight to the relationship between climate variability and phenotypic plasticity.

CHAPTER 2

METHODOLOGY

Study System

We studied the Panamanian slender anole (hereafter, "slender anole"), which is a semi-arboreal arthropod predator that was historically ubiquitous throughout the lowland tropical rain forests of Panama. Nevertheless, this species has experienced population declines associated with climate change (Stapley et al., 2015). Slender anoles are an ideal model organism to test these hypotheses because they are a tropical thermoconforming ectotherm, typically occur at high densities in nature and adjust well to captive settings.

Field collection and Processing

All lizards were hand caught in Soberanía National Park, Panama. In the short-term temperature change experiment, we caught 24 male lizards in July of 2017. For the longer-term thermal stress experiment, we caught 40 lizards (equal sex ratio) in June of 2019. We transported lizards to Gamboa, Panama, allowed them to acclimate to laboratory conditions for 48 hours, then measured snout-vent-length and mass. For the longer-term experiment, we marked each lizard with a unique code using visual implant elastomer to ensure easy identification throughout the experiments. From July 2017 through July 2019 we captured and recorded field active body temperatures of 1318 slender anoles at the same site. We used these data (mean field-active body temperature of 27.8° C) to compare temperatures for our treatments.

Gene expression response to short-term temperature change

We placed lizards into three Percival incubators set to a warm (32°C), control (28°C), or cool (18°C) treatment (n=8 per treatment). We selected 32°C, 28°C, and 18°C because they are

representative of warm, average, and very cool field active body temperatures which are experienced in the forest . We exposed an individual lizard to one of the treatments for two hours, then euthanized the animal (via decapitation) and sampled tissue from the brain, liver and muscle.

RNA was isolated from these tissues using a TRIzol reagent protocol and then complementary DNA libraries were created using a KAPA stranded mRNA-Seq Kit. RNA data, containing both sequence and abundance scores, were produced by NextSeq Illumina platform. Illumina FASTQ data and adapters were removed using the paired end mode of the command line tool Trimmomatic (Bolger, Lohse & Usadel 2014). FASTX-toolkit was used to produce statistics about the quality and the sequences of FASTQ files before mapping to the *Anolis carolinensis* reference genome because it is the closest relative with an assembled genome (Hannon 2010). BWA (Li & Durbin 2010) (Alföldi et al., 2011). Samtools was used to convert from sequence alignment map format (SAM) to compressed binary format (BAM), merge multiple sorted alignments into a single sorted file, index a coordinate-sorted BAM file and output a text file with sequence name, length and number of mapped reads (Li et al. 2009).

EdgeR was used to identify differentially expressed genes by conducting a pairwise analysis between the warm or cool treatment, and the control treatment for each sample (McCarthy, Chen, & Smyth, 2012; Robinson, McCarthy, & Smyth, 2010). Differentially expressed genes (DEGs) were identified as transcripts that had a false discovery rate (FDR) < 0.05. Beyond global gene expression, we scrutinized the expression of a priori selected candidate genes which are known to participate in the cellular response to heat (Table 2.1) (Jassal et al. 2020). A priori genes were identified as differentially expressed if they had a p-value less than 0.00067 (Bonferroni- correction). We used Gene Ontology (GO) analysis (Ashburner et al. 2000;

The Gene Ontology Consortium 2018) to identify the biological processes for which DEGs were most involved. Significantly enriched processes were identified as those that had an FDR < 0.05.

Phenotypic plasticity under longer-term thermal stress: trait measurement

We measured voluntary thermal maximum or VTmax by placing lizards in an incubator set to 50°C (Cowles 1944). VTmax is an estimate of upper thermal tolerance and was determined as the internal body temperature at which the individual displays obvious escape behavior. The critical thermal minimum (CTmin), an estimate of lower thermal tolerance, was determined as the temperature at which lizards cannot right themselves. CTmin was measured by cooling lizards for ten minutes to immobility in an incubator set to 2°C. Lizards were then removed from the incubator and allowed to warm towards ambient room temperature (~22°C). We flipped the lizard onto its back every 10 seconds during this process and recorded the internal body temperature at which the animal regained its righting response.

Preferred body temperature (Tpref) is a repeatable trait that gives insight into thermoregulatory behavior and activity times of organisms (Hertz, Huey & Stevenson 1993). Preferred body temperature is traditionally recorded in an artificial thermal gradient to determine the optimal temperature for physiological performance in the absence of ecological constraint (Angilletta 2009). Thermal gradients were constructed using four rectangular plastic bins (0.85m length □ 0.4m width □ 0.4m depth) with a substrate of soil (~1 cm deep). The warm end of the gradient was set using a 250-W infrared heat lamp suspended above the container. The cool end of the gradient was set by the ambient temperature of the room. The temperature gradient spanned 20°-36°C. Humidity has been shown to affect thermal preference (Crowley 1987), thus, we increased the humidity of the room to mimic environmental conditions by boiling 7.5 liters of water in a 15-liter stock pot (to generate steam). Lizards had a thermocouple (Type T) inserted in

the cloaca and secured by a small piece of medical tape. Thermocouples were connected to an 8-channel temperature data logger (OctTCTemp2000, Madgetech Inc., Warner, N.H., USA). The data logger was programmed to record lizard body temperatures every 30 seconds for 1.5 hours. Lizards were left undisturbed for the duration of the trial. During trials, lizards were given 30 minutes to acclimate to the gradient. Data collected in the remaining 60 minutes of the trial were retained to calculate the mean, range, interquartile range, and standard deviation of each individual, which we take as different thermoregulatory traits that combine to form an individual's thermal preference.

We measured snout-vent length and body mass before and after exposure to longer-term increasing temperatures and calculated growth in both SVL and mass units per day. We then used residual body mass from a linear regression model of body mass on snout-vent-length as an index of body condition. Following the longer-term experiment, we also dissected lizards and weighed organs associated with energy storage and reproduction, including visceral fat bodies, liver mass, and gonad mass.

Phenotypic plasticity under longer-term thermal stress: mesocosm design

Forty lizards were assigned to 40 9" x 9" x 18" mesh cages that were then placed inside of two temperature-controlled greenhouses inside the insectaries of the Smithsonian Tropical Research Institute in Panama. Each cage had a branch for perching and a leaf litter substrate such that lizards were able to avoid lethally high temperatures. Each greenhouse had a bucket of water to maintain humidity, the control had an average of 58% humidity while the heat had an average of 54% humidity. We monitored the temperature of one of the greenhouses (20 lizards) by setting the thermostat of a York Indoor Air Conditioner Unit (Model YNFFXC036BAAD-FX with cooling capacity 9070 kcal/h; 10.55 kW; 36000 Btu/h) to 24°C for five days, 25°C for five

days and 26°C for the remaining 21 days. The other 20 enclosures experienced warmer conditions by increasing the thermostat of the air conditioner unit from 24 to 30°C over a period of 14 days then holding the thermostat constant at 30°C for another 17 days (Figure 2.1). Here, we were mimicking the gradual onset of an environmental heat wave. Lizards were fed six crickets every three days and misted with water every day. After four weeks of treatment, thermal variables were measured again.

We monitored the temperature of lizards by taking 989 surface body measurements during the length of the experiment. Initially, we measured surface body temperatures using a Tacklife It-t04 digital infrared thermometer gun. Over a period of five days (control n=59, heat n=61), we compared measurements of the Tacklife gun to measurements taken with a Fluke-62 MAX infrared thermometer gun and internal body temperature measurements with an Omega HH-25KC cloacal thermometer by measuring the same lizards with all three instruments Figure 2.2. Following this assessment time period, we used the Fluke infrared thermometer to make all surface body measurements because it had less variance and more closely matched internal body temperatures than the Tacklife gun. We used surface body temperatures as our estimate of lizard temperature for the rest of the experiment because the measurement of surface temperatures does not require the handling of lizards. Stress from handling can alter behavior and affect experimental results. For results on the measurements of surface body temperature see Table 2.2.

Statistical methods

For morphological traits, we used linear regression models, including sex, treatment, and sex by treatment interactions. Body size covariates were included in models when appropriate. Differences between initial and final values for thermoregulation and thermal tolerance traits were determined using a repeated measures analysis of variance (ANOVA). See supplementary

material to view models that include the explanatory variables sex and mass (Table 2.3 and Table 2.4). Prior to analyses, we ensured that all variables fit the assumptions of statistical tests. All statistical analyses were completed in JMP v. 12.0 (JMP 2019).

Table 2.1 Candidate genes based on cellular response to heat taken from Reactome.

ENSEMBL ID	Gene	Description
ENSACAT00000003558.3	HSF3	heat shock factor protein 3
ENSACAT00000005026.3	HSPH1-201	heat shock protein family H (Hsp110) member 1
ENSACAT00000010854.2	HBE1-202	hemoglobin subunit epsilon 1
ENSACAT00000012207.2	HBE1-201	hemoglobin subunit epsilon 1
ENSACAT00000014269.3	HYOU1-201	hypoxia upregulated 1
ENSACAT00000000480.3	MTOR-201	Mechanistic target of rapamycin kinase
ENSACAT00000001041.3	NUP133-201	Nucleoporin 133
ENSACAT00000001117.3	HSPA14-201	Heat shock protein family A (Hsp70) member 14
ENSACAT00000001379.3	MAPK1-201	Mitogen-activated protein kinase 1
ENSACAT00000001750.3	TPR-201	Translocated promoter region, nuclear basket protein
ENSACAT00000002100.3	RANBP2-201	E3 SUMO-protein ligase RanBP2
ENSACAT00000002235.2	SERPINH1-201	Serpin family H member 1
ENSACAT00000004096.2	HSPB8-201	Heat shock protein family B (small) member 8
ENSACAT00000004158.3	NUP107-201	Nucleoporin 107
ENSACAT00000004164.2	NUP153-201	Nucleoporin 153
ENSACAT00000004172.3	HSPA5-201	Heat shock protein family A (Hsp70) member 5
ENSACAT00000004209.2	MRPL18-201	Mitochondrial ribosomal protein L18
ENSACAT00000004237.3	HSPA12B-201	Heat shock protein family A (Hsp70) member 12B
ENSACAT00000004906.2	HSPA8-201	Heat shock protein family A (Hsp70) member 8
ENSACAT00000004943.3	AKT1S1-201	AKT1 substrate 1
ENSACAT00000004958.3	ATR-201	ATR serine/threonine kinase
ENSACAT00000005026.3	HSPH1-201	Heat shock protein family H (Hsp110) member 1
ENSACAT00000005189.3	EP300-201	E1A binding protein p300
ENSACAT00000005297.2	novel transcript	ST13 Hsp70 interacting protein
ENSACAT00000005438.3	AAAS-201	Aladin WD repeat nucleoporin
ENSACAT00000006268.3	NUP85-201	Nucleoporin 85
ENSACAT00000006653.3	DNAJC2-201	DnaJ heat shock protein family (Hsp40) member C2
ENSACAT00000006822.3	NUP43-201	Nucleoporin 43
ENSACAT00000007584.3	NUP93-201	Nucleoporin 93
ENSACAT00000008057.3	HSBP1-201	Heat shock factor binding protein 1
ENSACAT00000008084.3	HDAC6-201	Histone deacetylase 6
ENSACAT00000008714.3	NUP35-201	Nucleoporin 35
ENSACAT00000008884.2	ATMIN-201	ATM interactor
ENSACAT00000008932.3	NDC1-201	NDC1 transmembrane nucleoporin
ENSACAT00000009163.3	PTGES3-201	Prostaglandin E synthase 3
ENSACAT00000009312.3	CAMK2G-201	Calcium/calmodulin dependent protein kinase II gamma
ENSACAT00000009436.3	NUP160-201	Nucleoporin 160
ENSACAT00000010084.3	RICTOR-201	Rapamycin-insensitive companion of mTOR
ENSACAT00000010135.3	HSPA12A-201	Heat shock protein family A (Hsp70) member 12A
ENSACAT00000010599.3	SIRT1-201	Sirtuin 1

ENSACAT00000011474.3	DNAJB6-201	DnaJ heat shock protein family (Hsp40) member B6
ENSACAT00000011568.3	NUP205-201	Nucleoporin 205
ENSACAT00000011882.2	HSPA4L-201	Heat shock protein family A (Hsp70) member 4 like
ENSACAT00000011891.3	VCP-201	Valosin containing protein
ENSACAT00000012302.3	COL4A6-201	Collagen type IV alpha 6 chain
ENSACAT00000012335.3	CAMK2B-201	Calcium/calmodulin dependent protein kinase II beta
ENSACAT00000012671.2	CREBBP-201	CREB binding protein
ENSACAT00000012678.3	HSF1-201	Heatsock factor 1
ENSACAT00000012710.2	NUP54-201	Nucleoporin 54
ENSACAT00000012920.3	NUP210-201	Nucleoporin 210
ENSACAT00000013076.2	RPA3-201	Replication protein A3
ENSACAT00000013341.2	SEH1L-201	SEH1 like nucleoporin
ENSACAT00000013423.3	TNFRSF21-201	TNF receptor superfamily member 21
ENSACAT00000013752.3	NUPL2-201	Nucleoporin like 2
ENSACAT00000013773.2	MLST8-201	MTOR associated protein, LST8 homolog
ENSACAT00000014093.3	NUP98-201	Nucleoporin 98
ENSACAT00000014210.3	NUP62-201	Nucleoporin 62 C-terminal like
ENSACAT00000014639.3	RPA1-201	Replication protein A1
ENSACAT00000014893.3	HSP90AB1-201	Heat shock protein 90 alpha family class B member 1
ENSACAT00000015126.3	MAPK3-201	Mitogen-activated protein kinase 3
ENSACAT00000015291.3	FKBP4-201	FKBP prolyl isomerase 4
ENSACAT00000015477.3	NUP88-201	Nucleoporin 88
ENSACAT00000015743.3	NUP50-201	Nucleoporin 50
ENSACAT00000015808.1	HSPA2-201	Heat shock protein family A (Hsp70) member 2
ENSACAT00000016013.3	HSPA9-201	Heat shock protein family A (Hsp70) member 9
ENSACAT00000016265.3	RAE1-201	Ribonucleic acid export 1
ENSACAT00000016605.3	NUP37-201	Nucleoporin 37
ENSACAT00000017032.3	DNAJC7-201	DnaJ heat shock protein family (Hsp40) member C7
ENSACAT00000017077.3	DNAJB1-201	DnaJ heat shock protein family (Hsp40) member B1
ENSACAT00000017108.3	HSPBP1-201	HSPA (Hsp70) binding protein 1
ENSACAT00000017313.2	AHSA1-201	activator of HSP90 ATPase activity 1
ENSACAT00000017726.3	RPA2-201	Replication protein A2
ENSACAT00000028911.2	novel transcript	Nuclear pore complex protein Nup214
ENSACAT00000029150.1	DEDD2-201	death effector domain containing 2
ENSACAT00000029629.1	LOC100557088	cholinesterase
ENSACAT00000022143.2	LOC100561946	cholinesterase

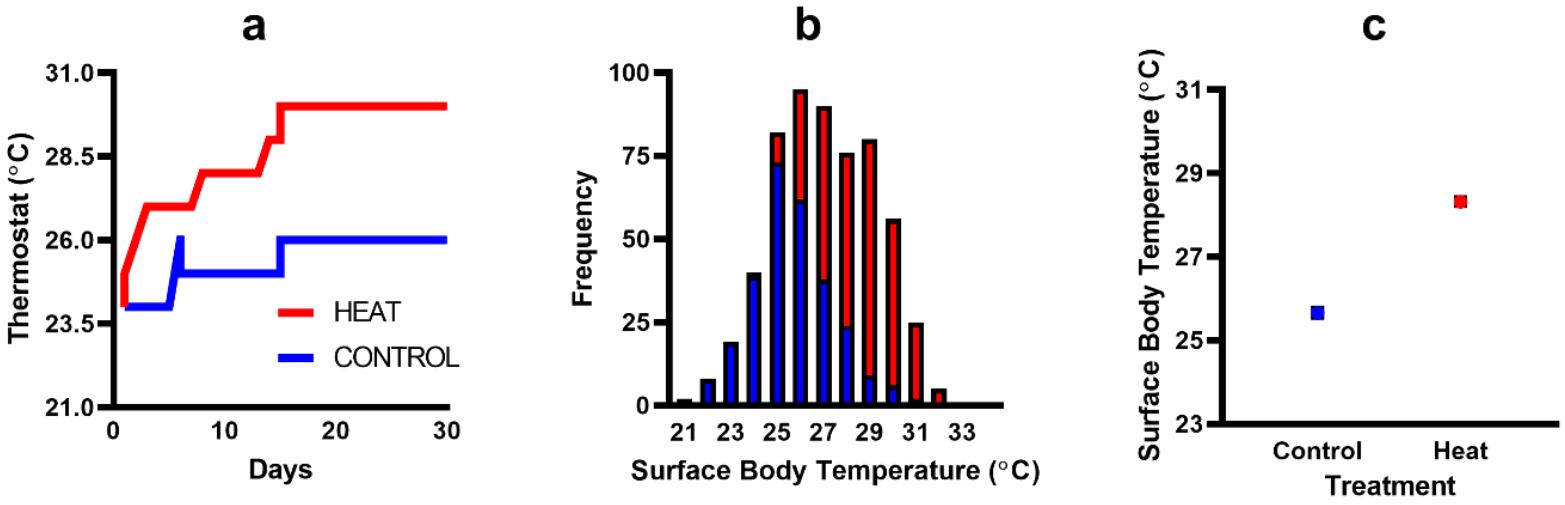

Figure 2.1 a) Thermostat setting of an indoor air conditioner unit during the long-term experiment. b) Frequency distribution of surface body temperatures of control (blue) and heat-stressed (red) lizards measured with a fluke infrared gun. c) Mean ± SE surface body temperature measured with a fluke infrared gun.

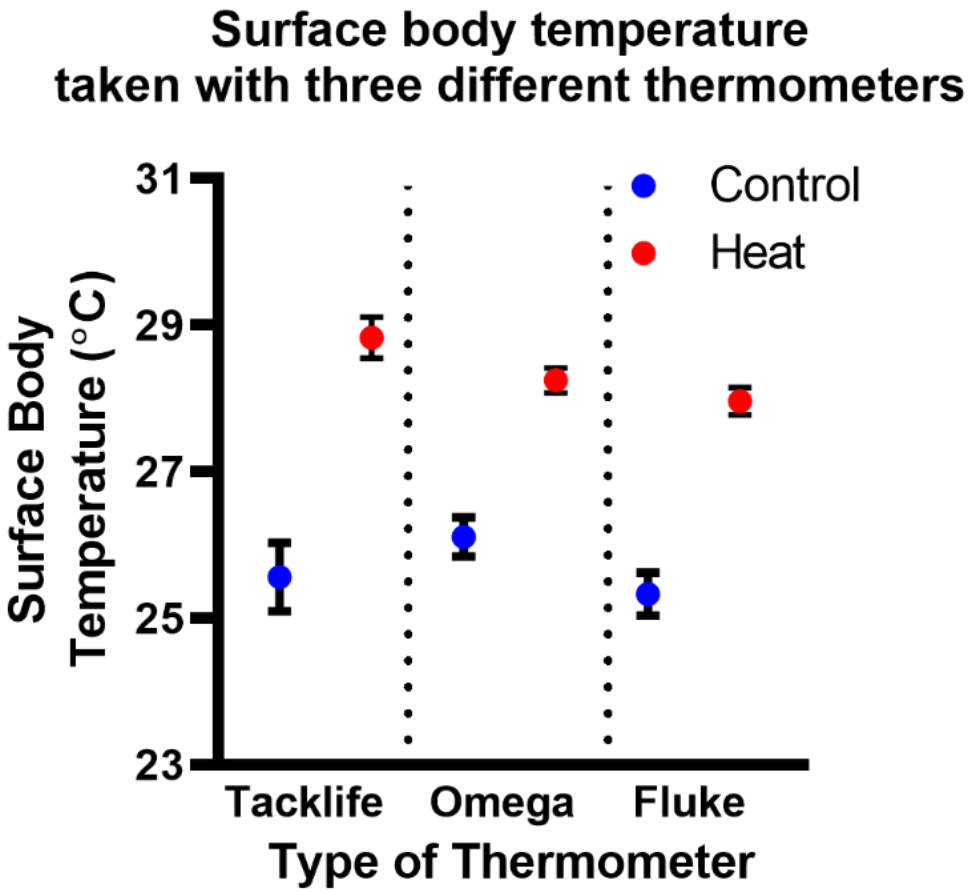

Figure 2.2 Surface body temperatures taken with three different thermometers over a period of four days. Following this assessment period, we used the Fluke infrared gun because The Tacklife infrared gun was more variable than either the Omega cloacal thermometer or the Fluke infrared gun. We used surface body temperatures as our estimate of lizard temperature for the rest of the experiment because the measurement of surface temperatures does not require the handling of lizards.

Table 2.2 Surface body temperatures taken with Tacklife infrared thermometer, Omega thermocouple or Fluke infrared thermometer.

Date Range	Statistic	Tacklife	Thermocouple	Fluke	Measurements	Treatment	Thermostat
5/25/2019-6/12/2019	Mean	24.9	NA	NA	210	CONTROL	23-25
5/25/2019-6/12/2019	Median	24.7	NA	NA	210	CONTROL	23-25
5/25/2019-6/12/2019	Range	16.5	NA	NA	210	CONTROL	23-25
5/25/2019-6/12/2019	Variance	10.9	NA	NA	210	CONTROL	23-25
5/25/2019-6/12/2019	Mean	27.4	NA	NA	212	HEAT	23-28
5/25/2019-6/12/2019	Median	27.6	NA	NA	212	HEAT	23-28
5/25/2019-6/12/2019	Range	19.3	NA	NA	212	HEAT	23-28
5/25/2019-6/12/2019	Variance	10.7	NA	NA	212	HEAT	23-28
6/8/2019-6/12/2022	Mean	25.6	26.1	25.3	59	CONTROL	25
6/8/2019-6/12/2022	Median	24.9	25.5	25.0	59	CONTROL	25
6/8/2019-6/12/2022	Range	15.0	9.0	12.1	59	CONTROL	25
6/8/2019-6/12/2022	Variance	12.9	4.3	5.0	59	CONTROL	25
6/8/2019-6/12/2022	Mean	28.8	28.2	28.0	61	HEAT	28
6/8/2019-6/12/2022	Median	28.8	28.2	28.1	61	HEAT	28
6/8/2019-6/12/2022	Range	10.4	6.5	6.8	61	HEAT	28
6/8/2019-6/12/2022	Variance	4.7	1.8	2.1	61	HEAT	28
6/14/2019-7/12/2019	Mean	NA	NA	26.0	187	CONTROL	26
6/14/2019-7/12/2019	Median	NA	NA	26.0	187	CONTROL	26
6/14/2019-7/12/2019	Range	NA	NA	10.8	187	CONTROL	26
6/14/2019-7/12/2019	Variance	NA	NA	3.3	187	CONTROL	26
6/14/2019-7/12/2019	Mean	NA	NA	29.0	264	HEAT	30
6/14/2019-7/12/2019	Median	NA	NA	29.1	264	HEAT	30
6/14/2019-7/12/2019	Range	NA	NA	7.5	264	HEAT	30
6/14/2019-7/12/2019	Variance	NA	NA	2.0	264	HEAT	30

Table 2.3 Results are from two-way repeated measures ANOVA from means of both treatments with interaction. For thermal limits $\alpha = 0.05$ while for thermal preference $\alpha = 0.0083$.

	Treatment (between treatments)			Time (within treatments)		Time*Treatment (within treatments)	
	d.f.	F	P	F	P	F	P
VT_{max}	1,35	0.0176434	0.4373	10.7958	**0.0023**	3.22	0.0814
CT_{min}	1,35	0.4165	0.5229	5.2552	**0.0280**	0.5995	0.4440
T_{pref} mean	1,33	0.3075	0.5829	4.8730	0.0343	0.2168	0.6446
T_{pref} max	1,33	0.7815	0.3831	0.0202	0.8878	0.2140	0.6467
T_{pref} min	1,33	0.0002	0.9882	5.8205	0.0216	1.2875	0.2647
T_{pref} 50	1,33	0.3032	0.5856	5.3744	0.0268	0.2054	0.6533
T_{pref} SD	1,33	2.9989	0.0927	7.1165	0.0117	0.3275	0.5710
T_{pref} Range	1,33	0.9456	0.3379	4.0008	0.0538	0.4300	0.5166

Table 2.4 Four-way models including sex, mass, treatment and time for preferred body temperature and thermal limits. For thermal limits α = 0.05 while for thermal preference α = 0.0125.

			Sex (between)	
	d.f.		F	P
VT_{max}	1,31	Sex	2.6307	0.1149
	1,31	Treatment	0.4213	0.5211
	1,31	Final Mass	0.0531	0.8192
	1,31	Time	5.0056	**0.0326**
	1,31	Time*Sex	0.0043	0.9479
	1,31	Time*Treatment	2.5402	0.1211
	1,31	Time*Final Mass	2.918	0.0976
	3,31	All Between	1.2267	0.3166
	3,31	All Within Interactions	1.9906	0.1359
CT_{min}	1,31	Sex	4.2899	**0.0468**
	1,31	Treatment	0.215	0.6461
	1,31	Final Mass	0.0079	0.9297
	1,31	Time	0.0459	0.8318
	1,31	Time*Sex	0.1872	0.6683
	1,31	Time*Treatment	0.2775	0.6021
	1,31	Time*Final Mass	0.0225	0.8817
	3,31	All Between	1.5817	0.2137
	3,31	All Within Interactions	0.1756	0.9121
T_{pref} mean	1,29	Sex	0.3484	0.5596
	1,29	Treatment	0.7893	0.3816
	1,29	Final Mass	3.1475	0.0865
	1,29	Time	0.7134	0.4052
	1,29	Time*Sex	0.2098	0.6504
	1,29	Time*Treatment	0.4416	0.5116
	1,29	Time*Final Mass	0.1525	0.699
	3,29	All Between	1.2515	0.3093
	3,29	All Within Interactions	0.1525	0.699
T_{pref} max	1,29	Sex	0.2097	0.6505
	1,29	Treatment	0.8721	0.3581
	1,29	Final Mass	2.9154	0.0984
	1,29	Time	0.8909	0.353
	1,29	Time*Sex	0.845	0.3656
	1,29	Time*Treatment	0.8568	0.3623

	1,29	Time*Final Mass	1.0531	0.3133
	3,29	All Between	1.1892	0.3311
	3,29	All Within Interactions	0.8051	0.5013
T_{pref} min	1,29	Sex	1.0884	0.3054
	1,29	Treatment	0.1442	0.7069
	1,29	Final Mass	2.3246	0.1382
	1,29	Time	4.1985	0.0496
	1,29	Time*Sex	0.0892	0.7673
	1,29	Time*Treatment	1.2644	0.27
	1,29	Time*Final Mass	2.4476	0.1286
	3,29	All Between	0.96	0.4248
	3,29	All Within Interactions	1.1526	0.3446
T_{pref} Range	1,29	Sex	0.0779	0.7821
	1,29	Treatment	0.5186	0.4772
	1,29	Final Mass	0.4959	0.4869
	1,29	Time	6.9743	0.0132
	1,29	Time*Sex	0.9549	0.3366
	1,29	Time*Treatment	0.1263	0.7248
	1,29	Time*Final Mass	5.053	0.0324
	3,29	All Between	0.372	0.7738
	3,29	All Within Interactions	1.7559	0.1776

CHAPTER 3

RESULTS

Gene expression in response to short-term temperature change

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) compared to 18°C (cool treatment). In the brain, relative to the control treatment, there were 5322 genes differentially expressed in response to the warm treatment, but only one gene differentially expressed in response to the cool treatment (Figure 3.1, brain). A similar trend was found in the liver (59 genes expressed in response to warm treatment, 7 genes expressed in response to cool treatment) and in the muscle (33 genes expressed in response to the warm treatment, 12 genes expressed in response cool temperature) (Figure 3.1, Liver, Muscle).

There were many more genes upregulated than down regulated in response to warm temperature. In the brain we found 3688 genes upregulated and 1634 genes downregulated, with a similar trend in the liver (49 genes upregulated, 10 downregulated) and the muscle (19 genes upregulated, 14 downregulated).

Similarly, we found that the magnitude of the transcriptomic response measured by average log fold change of all DEGs, regardless of tissue, was greater in response to warm treatment than in response to cool treatment. In the brain, the magnitude (average log fold change) of DEGs in response to warm treatment was greater than the magnitude of DEGs in response to the cool treatment (Figure 3.2), with a similar trend in liver and muscle tissue. 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 (Figure

3.2). There was a positive correlation between the gene expression response to warm treatment and the gene expression response to cool treatment, indicating that the same genes that were upregulated in response to warm treatment were also upregulated in response to cool treatment (Figure 3.3). However, these genes differ in their magnitude of expression between the warm and cool treatment as stated above.

All three tissues exhibited DEGs from our a priori selected pathway, the cellular response to heat. We describe each of these DEGs, the majority of which belonged to heat shock protein families, in Table 3.1. The pattern of DEGs that participate in cellular response to heat, was the same as that found in global gene expression (Figure 3.4 and Figure 3.5)

Differentially expressed genes in the liver and muscle belonged to biological processes that protect from protein degradation (Table 3.2). We predicted an adaptive gene expression response would be characterized by heat shock response. Differentially expressed genes from liver represented four significant Gene Ontology (GO) terms, two of which were consistent with our hypothesis: protein folding (GO:0006457) and response to heat (GO:0009408). Genes related to the Hsp40, Hsp70, and Hsp90 families were represented in both GO terms. Differentially expressed genes from muscle represented three significant GO terms, one of which was consistent with our hypothesis: protein folding (GO:0006457). Similarly, genes related to the Hsp40, Hsp70, and Hsp90 families were represented in this biological process as well. The brain had 112 significantly enriched biological processes with no processes related to the response to heat. However, protein ubiquitination (GO:0016567) was a top term which may indicate an increase of protein degradation.

Growth and body condition under longer-term thermal stress

We found that some measures of growth and energy storage differed between longer-term thermal stress. Lizards in the heat treatment grew more rapidly in SVL than lizards in the control treatment (Treatment, F3,30=5.2806, P=0.0287, Sex, F3,30=0.2425, P=0.6260, Treatment by Sex, F3,30=0.4311, P=0.5165), whereas growth estimated as mass gain did not differ between longer-term treatments (Treatment, F3,30=1.2959, P=0.2640, Sex, F3,30=0.9495, P=0.3376, Treatment by Sex, F3,30=0.1016, P=0.7521). Final body condition did not vary between treatments, although females had higher body condition than males across both treatments (Treatment, F3,30=0.2395, P=0.6281, Sex, F3,30=6.0863, P=0.0196, Treatment by Sex, F3,30=0.0016, P=0.9688). We found that visceral fat body mass differed between treatments in a sex-dependent fashion (Treatment, F4,30=0.6679, P=0.4168, Sex, F4,30=3.7484, P=0.0623, Treatment by Sex, F4,30=5.2999, P=0.0284, Body Mass, F4,30=5.1794, P=0.0302). Control females had larger fat bodies compared to heat-stressed females, while the opposite was true for males (larger fat bodies in heat males). Liver mass did not vary between treatments, although females had larger livers than males across both treatments (Treatment, F4,29=0.0074, P=0.9321, Sex, F4,29=4.8079, P=0.0365, Treatment by Sex, F4,29=0.1380, P=0.7130, Body Mass, F4,29=10.0177, P=0.0036). The size of the gonads did not differ between treatments for either females (Treatment, F2,13=2.5568, P=0.1338, Body Mass, F2,13=5.2283, P=0.0396) or males (Treatment, F2,15=0.7478, P=0.4008, Body Mass, F2,15=5.4034, P=0.0345).

Plasticity of thermal limits in response to longer-term thermal stress

We found that upper, but not lower, thermal limits were plastic in slender anoles. There was a significant increase in VTmax in response to longer-term heat stress (F1,17=10.23, P=0.0053) but not in response to the control treatment (F1,18=1.4568, P=0.2431). Average

CTmin decreased in both treatments but this change was not significant (control, $F_{1,18}=1.408$, $P=0.2513$, heat, $F_{1,17}=3.9265$, $P=0.639$) (Figure 3.6).

Plasticity of thermoregulatory behavior in response to longer-term thermal stress

We detected limited plasticity of thermoregulatory behavior in response to longer-term thermal stress in the slender anole. We did not find a significant change in the mean (heat: $F_{1,17}=1.2869$, $P=0.2724$; control: $F_{1,16}=4.4933$, $P=0.05$) or maximum (heat: $F_{1,17}=0.0560$, $P=0.8158$; control: $F_{1,16}=0.1678$, $P=0.0.6875$) body temperatures chosen in a thermal gradient in response to either the treatments. By contrast, the minimum temperature chosen in a thermal gradient decreased significantly in response to the control ($F_{1,16}=13.9098$, $P=0.0018$), but not the heat ($F_{1,17}=0.5503$, $P=0.4683$), treatment (Table 3.3 and Figure 3.7).

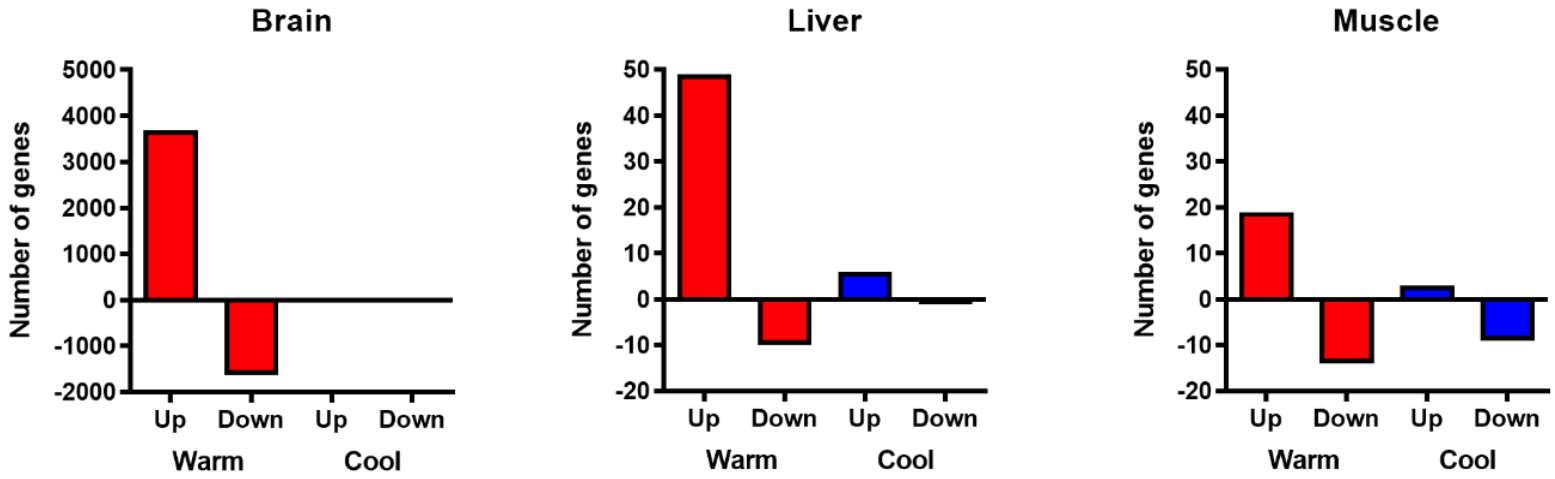

Figure 3.1 All three tissues adaptively shifted gene expression by expressing a greater number of genes in response to the warm treatment. DEGs were identified by performing a pairwise analysis between either the warm or cool treatment and the control treatment. Genes were considered differentially expressed if they had an FDR < 0.05.

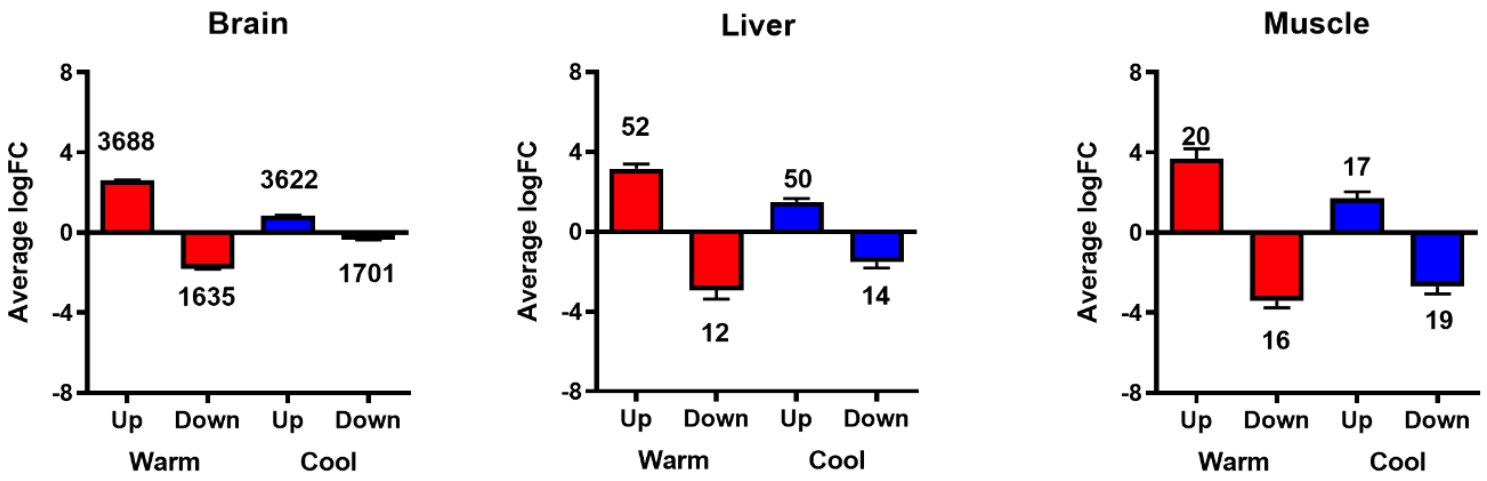

Figure 3.2 The magnitude of the transcriptomic response across all three tissues is greater in response to the warm treatment than in response to the cool treatment. The magnitude of response is quantified as the average log fold change response to the warm or cool treatment for all DEGs.

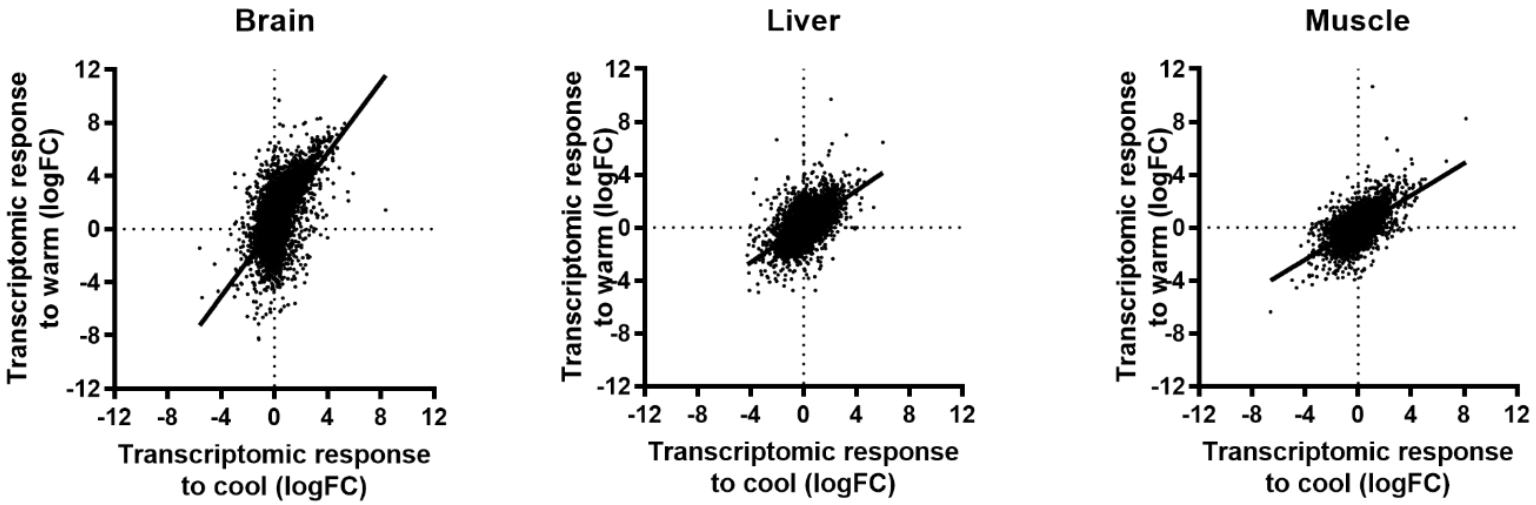

Figure 3.3 Genes upregulated in response to the warm treatment were also upregulated in response to the cool treatment. Each point represents one gene and a trendline indicates the positive relationship between genes transcribed in response to warm or cool treatment relative to the control treatment.

Table 3.1 A priori selected DEGs that participate in the cellular response to heat pathway. Negative Log_2FC values are downregulated genes in response to heat treatment and positive values are upregulated genes. Bolded genes were identified as differentially expressed in all three tissues. Genes are ordered from most to least significant. $\alpha = 0.00067$ (Bonferroni- correction)

Tissue	ENSEMBL ID	Gene	Description	Log_2FC	log_2CPM
brain	**ENSACAT00000017077**	**DNAJB1-201**	**DnaJ heat shock protein family (Hsp40) member B1**	**2.46**	**7.40**
	ENSACAT00000015808	**G1KRK2**	**Heat shock-related 70 KDA protein 2**	**7.89**	**9.48**
	ENSACAT00000004164	NUP153-201	Nucleoporin 153	3.51	3.97
	ENSACAT00000028911	NUP214	Nuclear pore complex protein Nup214	3.39	3.19
	ENSACAT00000014893	HSP90AB1-201	Heat shock protein 90 alpha family class B member 1	3.28	8.98
	ENSACAT00000012678	HSF1-201	Heatsock factor 1	3.49	3.25
	ENSACAT00000012710	NUP54-201	Nucleoporin 54	3.14	4.16
	ENSACAT00000004906	HSPA8-201	Heat shock protein family A (Hsp70) member 8	3.81	8.20
	ENSACAT00000017313	AHSA1-201	activator of HSP90 ATPase activity 1	3.60	6.38
	ENSACAT00000011568	NUP205-201	Nucleoporin 205	-1.90	5.44
	ENSACAT00000008057	HSBP1-201	Heat shock factor binding protein 1	3.61	5.08
	ENSACAT00000009163	PTGES3-201	Prostaglandin E synthase 3	2.37	5.45
	ENSACAT00000011882	HSPA4L-201	Heat shock protein family A (Hsp70) member 4 like	2.16	5.40
	ENSACAT00000009436	NUP160-201	Nucleoporin 160	-1.49	4.32
	ENSACAT00000017108	HSPBP1-201	HSPA (Hsp70) binding protein 1	1.97	4.99
	ENSACAT00000013341	SEH1L-201	SEH1 like nucleoporin	3.12	3.30
	ENSACAT00000001041	NUP133-201	Nucleoporin 133	-1.60	5.74
	ENSACAT00000008884	ATMIN-201	ATM interactor	2.20	2.76
	ENSACAT00000015291	FKBP4-201	FKBP prolyl isomerase 4	2.17	6.93
	ENSACAT00000006822	NUP43-201	Nucleoporin 43	-1.25	4.78
	ENSACAT00000007584	NUP93-201	Nucleoporin 93	2.26	3.43
liver	ENSACAT00000014210	NUP62-201	Nucleoporin 62 C-terminal like	1.86	4.76
	ENSACAT00000017077	**DNAJB1-201**	**DnaJ heat shock protein family (Hsp40) member B1**	**4.07**	**7.40**
	ENSACAT00000015808	**G1KRK2**	**Heat shock-related 70 KDA protein 2**	**9.72**	**9.48**
	ENSACAT00000011882	HSPA4L-201	Heat shock protein family A (Hsp70) member 4 like	2.19	5.40
	ENSACAT00000004906	HSPA8-201	Heat shock protein family A (Hsp70) member 8	2.59	8.20
muscle	**ENSACAT00000017077**	**DNAJB1-201**	**DnaJ heat shock protein family (Hsp40) member B1**	**4.21**	**7.40**
	ENSACAT00000015808	**G1KRK2**	**Heat shock-related 70 KDA protein 2**	**6.76**	**9.48**

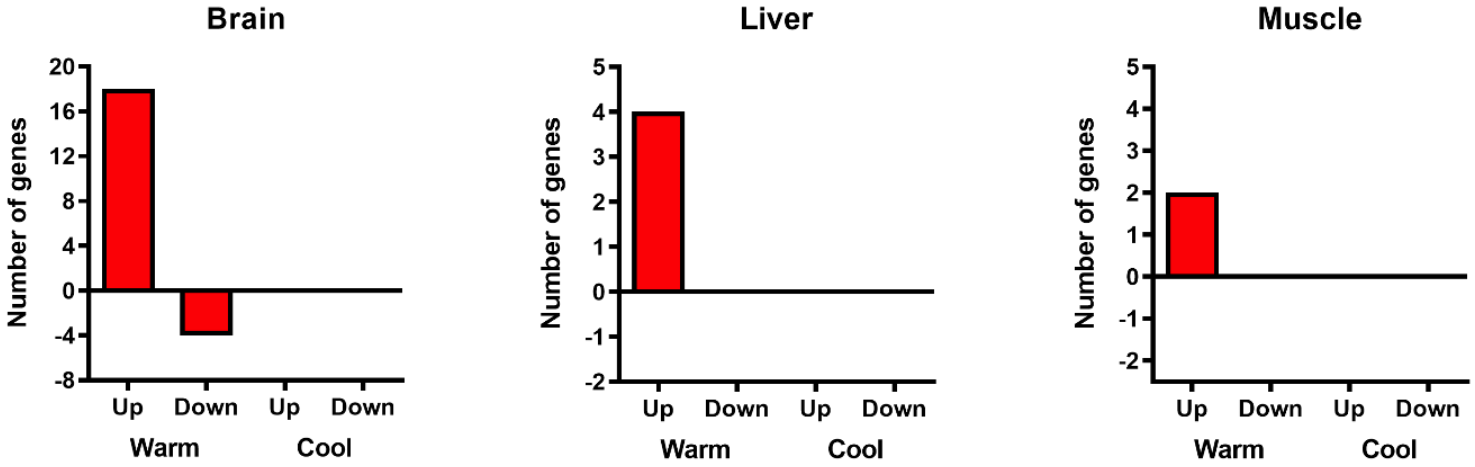

Figure 3.4 All three tissues adaptively shifted gene expression by expressing genes that participate in the cellular response to heat shock. There are a greater number of DEGs in response to warm treatment than in response to cool treatment. DEGs were identified by performing a pairwise analysis between either the warm or cool treatment and the control treatment. Genes from this network were considered differentially expressed if they had a p-value less than 0.00067 (Bonferroni- correction). These patterns of expression parallel what we found for global gene expression.

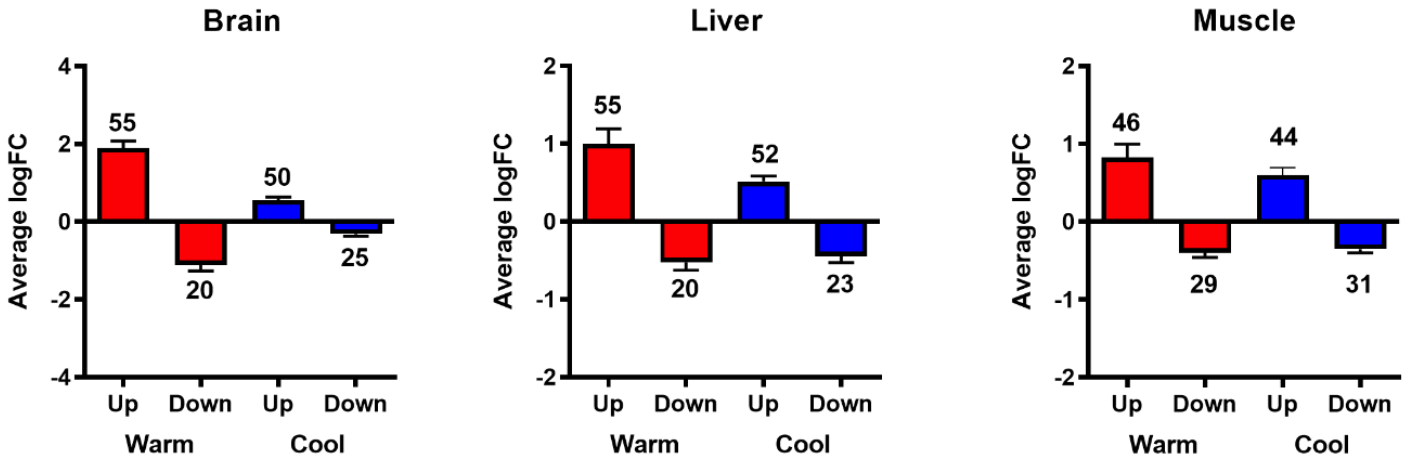

Figure 3.5 All three tissues adaptively responded to warm temperature by expressing a greater magnitude of transcripts that participate in cellular response to heat. The magnitude of transcriptomic response is quantified as the average log fold change of DEGs from the cellular response to heat network. These patterns of expression parallel what we found for global gene expression.

Table 3.2 DEGs from liver and muscle represented four and three significantly enriched GO terms, respectively. The biological processes protein folding (GO:0006457) and response to heat (GO:0009408) were significantly enriched when we analyzed 55 DEGs from the liver and 31 DEGs from the muscle. Heat shock proteins represented some of the most highly expressed transcripts.

GO Term	Transcript ID	Gene	Tissue	Panther Family
GO:0006457 Protein Folding	ENSACAT00000000159	H9G3B6	liver & muscle	HEAT SHOCK PROTEIN HSP 90-ALPHA-RELATED
	ENSACAT00000015808	G1KRK2		HEAT SHOCK-RELATED 70 KDA PROTEIN 2
	ENSACAT00000017077	G1KTF1		DNAJ HOMOLOG SUBFAMILY B MEMBER 1
	ENSACAT00000006050	H9GAW2		DNAJ HOMOLOG SUBFAMILY A MEMBER 4
	ENSACAT00000014467	G1KPW3		CYSTEINE AND HISTIDINE-RICH DOMAIN-CONTAINING PROTEIN 1
GO:0009408 Response to heat	ENSACAT00000000159	H9G3B6	liver	HEAT SHOCK PROTEIN HSP 90-ALPHA-RELATED
	ENSACAT00000015808	G1KRK2		HEAT SHOCK-RELATED 70 KDA PROTEIN 2
	ENSACAT00000006050	H9GAW2		DNAJ HOMOLOG SUBFAMILY A MEMBER 4

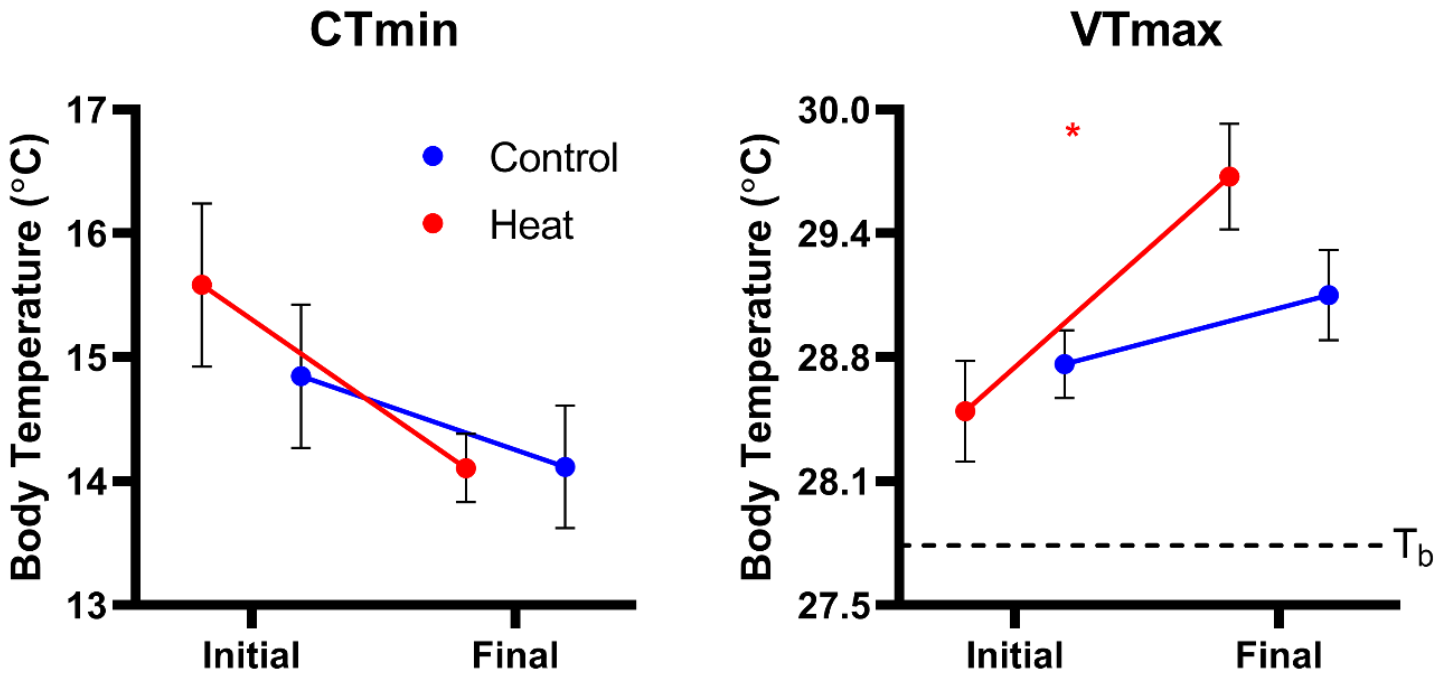

Figure 3.6 Mean CT_{min} (a) and VT_{max} (b) measured before (initial) and after (final) exposure to a control or heat-stress treatment. Difference between means was determined using a one-way repeated measures ANOVA with time as an explanatory variable and treatments calculated independently.

Table 3.3 Thermal Preference – Results are from one-way repeated measures ANOVA from means of each treatment independently. $\alpha = 0.0083$

	Control			Heat		
	d.f.	F	P	d.f.	F	P
Mean	1,16	4.4933	0.05	1,17	1.2869	0.2724
Maximum	1,16	0.1678	0.6875	1,17	0.056	0.8158
Minimum	1,16	13.9098	**0.0018**	1,17	0.5503	0.4683
T_{pref} 50	1,16	3.9594	0.064	1,17	1.6992	0.2098
Standard Deviation	1,16	5.3879	0.0338	1,17	2.1528	0.1606
Range	1,16	5.3275	0.0347	1,17	0.6967	0.4155

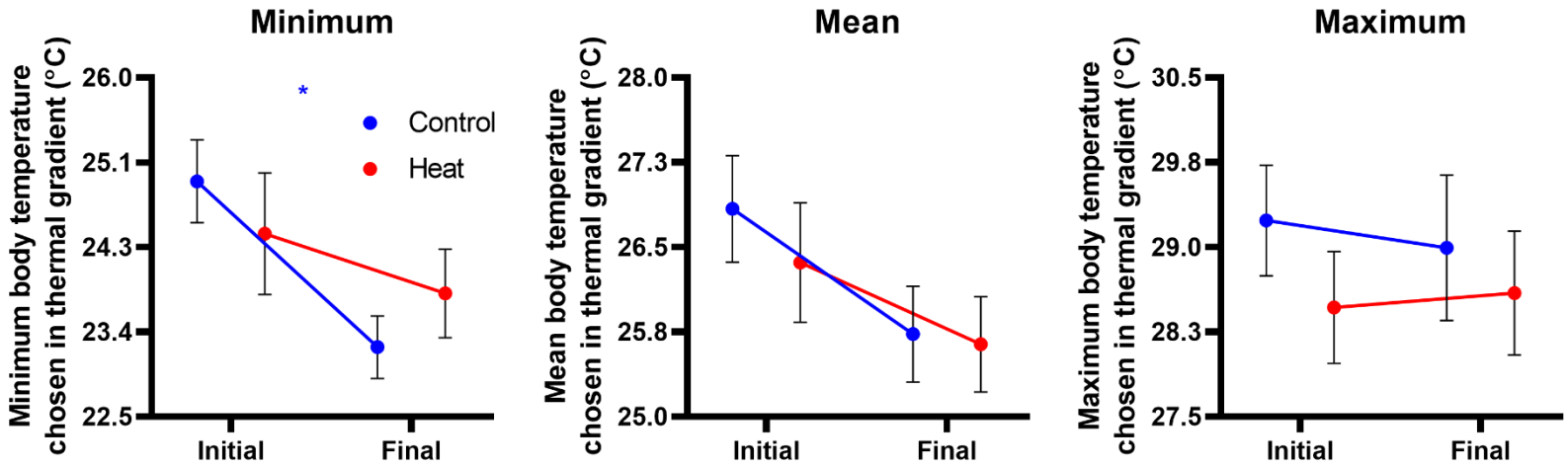

Figure 3.7 Minimum, mean, and maximum preferred body temperature chosen in a thermal gradient before initial treatment and after final treatment for the control and heat treatments. Difference between means was determined using a one-way repeated measures ANOVA with time as an explanatory variable and treatments calculated independently.

CHAPTER 4

DISCUSSION

We found evidence of adaptive gene expression patterns and adaptive phenotypic plasticity in response to temperature stress in the slender anole. Previous studies have suggested that terrestrial ectotherms inhabiting the tropics should have restricted plasticity in thermal physiology (Seebacher, White & Franklin 2015), and that the capacity for plasticity to buffer ectotherms from climate change is limited (Seebacher, White & Franklin 2014; Gunderson & Stillman 2015) . In particular, tropical forest lizards are predicted to be negatively impacted by climate change (Huey 2009; Sinervo et al. 2010). However, our results suggest that the slender anole might be capable of using plasticity to respond to climate change. For example, in the short-term experiment (two hours), three vital organs adaptively shifted gene expression during exposure to warm temperature. Differentially expressed genes (DEGs) included proteins that participate in heat shock response. Heat shock proteins likely play a role in phenotypic plasticity that we measured in response to longer-term thermal stress. During the longer-term experiment, slender anoles exposed to heat stress responded adaptively by increasing voluntary thermal maximum and energy stores in males.

We found that lizards upregulated genes that code for heat shock proteins genes in response to short-term temperature change. Heat shock proteins (Hsps) serve many biological functions but are most well-known for their role in heat shock response (Ritossa 1962) and their expression is used to infer how organisms respond to climate change (Tomanek 2010; González et al. 2016). Two important families of heat shock proteins are Hsp90 and Hsp70, which are highly conserved across eukaryotes and bacteria (Takayama, Xie & Reed 1999; Johnson 2012). We identified DEGs of three families of heat shock proteins (Hsp70, Hsp40, and Hsp90) in

brain, liver and muscle and found biological processes in the liver and muscle that were significantly enriched in response to heat stress in slender anoles. Biological processes in the brain that were most enriched included catabolic pathways, protein ubiquitination, and protein transport, although this should be interpreted cautiously because there were 112 significant terms. Hsp90 primarily binds native proteins (Jakob et al. 1999) and has evolved to function with a large number of co-chaperones (Pearl & Prodromou 2006) while Hsp70 can refold aggregated proteins, aid the assembly of newly produced proteins, prevent the clustering of unfolded proteins, and depends on Hsp40/DnaJ (Mayer & Bukau 2005). These two protein families interact in some pathways where Hsp70 acts as a co-chaperone by transferring unfolded proteins to Hsp90 (Wegele, Müller & Buchner 2004). Many eukaryotes exposed to heat shock upregulate both Hsp90 (Millson et al. 2007; Liu et al. 2013; Akashi et al. 2016; Qian & Xue 2016; Huang et al. 2018) and Hsp70 (Zhang et al. 2002; Hamdoun, Cheney & Cherr 2003; Akashi et al. 2016; Huang et al. 2018) indicating that adaptive gene expression in response to elevated temperatures could be associated with regulatory elements of heat shock proteins. Indeed, an increase in concentration of heat shock transcription factor 1 (HSF1) is linked to plasticity of heat shock response in the fish, *Gillichthys mirabilis* (Buckley & Hofmann 2002).

The results from our short-term experiment indicate tropical forest lizards adaptively shift patterns of gene expression in the liver, muscle, and brain in response to warm temperatures. Heat shock proteins play an important role in maintaining the proteome in response to immediate thermal stress and likely participate in the phenotypic plasticity measured in our longer-term experiment.

Beyond gene expression responses to short-term temperature change, we also found phenotypic plasticity in response to longer-term thermal stress. We documented a significant

increase in the voluntary thermal maximum in response to one month of thermal stress, but no change in the critical thermal minimum for either control or heat-stressed lizards. Previous literature has found that the plasticity of thermal limits, specifically CTmax, is constrained in terrestrial ectotherms and may do little to buffer organisms from the changes in temperature that will occur due to climate change (Gunderson & Stillman 2015). However, our findings are consistent with the Bogert effect which posits that ectotherms that are relatively immobile must have more plasticity of physiological traits to compensate for the inability to thermoregulate (Huey, Hertz & Sinervo 2003). Although thermoconforming lizards are likely to display some thermoregulation (evidence of escape behavior used to identify VTmax), their environment has limited thermal space because of the thermal homogeneity under the canopy of lowland tropical rainforests. Our results suggest that phenotypic plasticity of thermal limits may aid the response of the slender anole during a future where heat waves are more common.

Behavioral thermoregulation does not appear to be plastic in the slender anole. We did not find a significant change in preferred body temperature in response to either treatment, except for minimum temperature chosen in response to the control treatment. Although, we successfully increased the temperature of the heat treatment (Figure 1B), the control treatment was cooler than the average field active body temperatures. None of our other measures of behavioral thermoregulation changed in response to either longer-term temperature treatment (Table 5, S2, and S3). This indicates that even in the face of increasing temperatures, tropical lizards have limited capacity for plasticity of behavioral thermoregulation.

The role of plasticity in adaptation and long-term evolution is unclear (Ancel 2000; Price, Qvarnström & Irwin 2003; Ghalambor et al. 2007). Phenotypic plasticity is hypothesized to facilitate evolution by allowing population persistence and adaptive evolution to occur in future

generations. Our results suggest adaptive phenotypic plasticity may do the same in an environment experiencing climate change. We recorded adaptive phenotypic plasticity to warming temperature which indicates that processes that are hypothesized to facilitate evolution are likely to be crucial in the survival of species that are predicted to be negatively impacted by climate change. Further, evolution of plasticity can also allow population persistence (Chevin & Hoffmann 2017). Research in plasticity of gene expression has revealed genomic mechanisms behind adaptive plasticity (Campbell-Staton et al. 2020)(Kenkel & Matz, 2016), as well as pre and post transcriptional mechanisms including regulatory elements (Hauenschild et al. 2008), alternative splicing (Marden 2008) or histone modification that mediate phenotypic plasticity (Johannes et al. 2009). To further understand the implications of gene expression and phenotypic plasticity in the context of long-term evolution, future research should include multiple generations exposed to varying microclimates and should focus on genomic mechanisms that allow transgenerational modification of heat shock protein expression. Our work suggests that tropical forest lizards can alter gene expression patterns and thermal despite having evolved under thermally stable conditions. This indicates that 1) plasticity of gene expression and phenotypic plasticity should be considered when trying to predict the impact of climate change on tropical forest lizards and 2) regulatory pathways which mediate plasticity of gene expression will likely be under selection when plasticity allows organisms to persist.

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