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University of Nevada, Reno

**Mitochondrial haplotype influences the impact of simulated climate warming on a  
tropical ectotherm**

A thesis submitted in partial fulfillment  
of the requirements for the degree of

BACHELOR OF SCIENCE

by

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We recommend that the thesis  
prepared under our supervision by

**ERIKA NIETO**

entitled

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requirements for the degree of

**BACHELOR OF SCIENCE, BIOLOGY**

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## **ABSTRACT**

While the plight of polar bears in a rapidly warming world is much publicized, recent theory suggests that the organisms likely to be most at risk from rising temperatures are terrestrial arthropods and reptiles inhabiting the tropics. In such ectothermic species, metabolic rate increases exponentially with ambient temperature, and a small temperature increase in a warm environment therefore has a much larger physiological impact than does a comparable temperature increase in a cool environment. In studies recently conducted by my faculty mentors and their students on the Neotropical pseudoscorpion, *Cordylochernes scorpioides* (Zeh et al. 2012, 2014), it was found that simulated climate warming significantly decreased survival, average values for morphological traits, and level of sexual dimorphism. However, these effects were minor compared to catastrophic consequences for male fertility and female fecundity, pointing to reproduction as the life history stage that is most vulnerable to climate warming. Still to be investigated was the potential for a genetically-based response to elevated temperature. In the study reported here, I took advantage of the coexistence of two sympatric but highly divergent mitochondrial haplogroups in this pseudoscorpion to evaluate the potential impact of mitochondrial sequence variation on fitness traits and response to climate change. For survivorship and sperm production, my results reveal a significant interaction between mitochondrial haplotype and temperature treatment, thus demonstrating genotype-dependent effects and the potential for an adaptive evolutionary response to climate warming.

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## TABLE OF CONTENTS

Abstract.....	i
Acknowledgements.....	ii
Table of Contents.....	iii
Introduction.....	1
Materials and Methods.....	4
The Study Organism.....	4
Mitochondrial Haplotype Variation.....	8
Experimental Design.....	11
Morphometrics.....	13
Male Fertility Traits.....	14
Statistical Analyses.....	15
Results.....	16
Discussion.....	25
Literature Cited.....	28

## INTRODUCTION

In considering the potentially dire consequences of climate change, the plight of polar bears on rapidly contracting polar ice caps is much publicized and widely appreciated, and it is certainly true that higher latitudes, especially in the northern hemisphere, have experienced much greater increases in temperature than tropical regions. For example, surface temperatures in Alaska and the Yukon increased by 2.0-3.5 °C between 1970 and 2004, compared to only 0.2-1.0 °C in the tropics (Parry *et al.*, 2007). Despite the moderate 3.5-4.0°C increase in temperature predicted for the tropics by the end of the century (IPCC 2007), recent studies suggest that climate change may have its most severe physiological effects on tropical terrestrial ectotherms (Deutsch *et al.*, 2008; Dillon *et al.*, 2010). Adapted to already high and relatively constant temperatures, tropical ectotherms, with their narrow thermal safety margins, are likely to be particularly sensitive to climate warming (Addo-Bediako *et al.*, 2000; Deutsch *et al.*, 2008). In such ectothermic species, metabolic rate increases exponentially rather than linearly with ambient temperature (Gillooly *et al.*, 2001; Clark, 2006), and a small temperature increase in a warm environment therefore has a larger physiological impact than a large temperature increase in a cold environment (Dillion *et al.* 2010). If correct, this metabolic theory of climate warming has profound implications for global biodiversity, since tropical terrestrial arthropods constitute the vast majority of described animal species (Lewinsohn & Roslin, 2008).

While previous studies investigating acute heat stress and thermal tolerance in ectotherms (e.g., Klok *et al.*, 2004; Karl *et al.*, 2011; Zizzari & Ellers, 2011) have provided a foundation for understanding the physiological effects of climate warming, predicting how climate change will translate into fitness consequences for tropical arthropods requires an understanding of the effects of temperature increase on the entire life history of the species. To address this need, in 2011, the laboratory of my thesis advisors, Jeanne and David Zeh, carried out a simulated climate warming study of the effects of the predicted 3.5<sup>0</sup>C temperature increase on life history, morphological and reproductive traits in the Neotropical pseudoscorpion, *Cordylochernes scorpioides* (Zeh *et al.* 2012). Unlike most previous studies, this research was performed at fluctuating temperatures that closely mimicked the natural diurnal temperature cycle experienced by the species in the wild. The results of this investigation indicated that the phenotypic effects of elevated temperature are complex, differing between the sexes and life history stages. Although survival, sex ratio, morphological traits and level of sexual dimorphism were significantly affected by elevated temperature, these impacts were minor compared to the devastating consequences for reproduction. Reared at elevated temperature, males produced only 45% as many sperm as control males, while female fecundity and reproductive success were reduced to essentially zero. The findings thus suggest that reproduction may be the Achilles' heel of tropical ectotherms, as global climate change subjects these organisms to an increasingly hostile thermal environment.

The results of this previous research performed by the Zeh laboratory clearly points to reproduction as the life history stage of tropical arthropods that is by far the most



vulnerable to climate warming. However, the one-step increase in temperature imposed on *C. scorpioides* in this study provided no opportunity for adaptive evolution, and tropical species exhibit markedly higher levels of DNA sequence variation over small spatial scales than do temperate zone species (Wilcox *et al.*, 1997; Crawford, 2003; Hebert *et al.*, 2004; Clare, 2011; Strutzenberger *et al.*, 2011). In the absence of behavioral modifications that mitigate the effects of increased temperature or upslope migration to cooler altitudes (Colwell *et al.*, 2008), the best case scenario is that selection acting on this reservoir of genetic variation may enable some tropical ectotherms to overcome the disproportionately high metabolic costs they face as global climate continues to warm. What remains to be investigated is the extent to which genotypes vary in their response to elevated temperatures, and whether or not underlying genetic variation will enable tropical ectotherm species to respond adaptively to the inexorable rise in global temperature over the coming decades.

Given the fundamental role of mitochondria in metabolism, sequence variation in the mitochondrial genome that influences oxidative phosphorylation (OXPHOS) activity, mitochondrial protein translational efficiency, and/or mitochondrial cellular concentration could be an important determinant of the phenotypic and evolutionary responses of tropical ectotherms to rising temperatures. In *C. scorpioides*, sequencing of the mitochondrial ND2 gene has revealed that two highly divergent haplogroups (A and B2) coexist in central Panamá (Zeh *et al.* 2012). The two clades exhibit 15 to 18 amino acid differences in ND2 sequence, and approximately 7.5% divergence, involving more than 1,000 nucleotide substitutions across the entire genome (Fig. 1). The coexistence of two

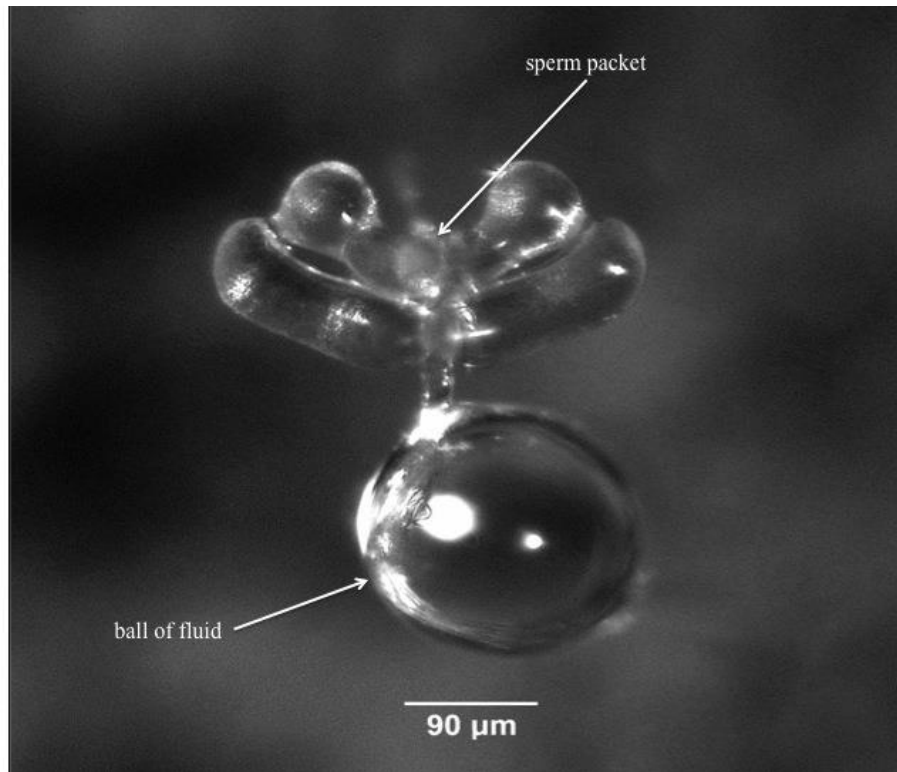
sympatric but highly divergent mitochondrial haplogroups in this pseudoscorpion provides an unparalleled opportunity to evaluate the impact of mitochondrial sequence variation on fitness traits and response to climate change. Pseudoscorpions in our population have been maintained in the laboratory for a minimum of 14 generations, in which they were mated randomly with respect to mitochondrial haplotype. Consequently, the A and B2 mitochondrial clades are nearly homogeneous with respect to nuclear genetic background, greatly increasing the likelihood that any systematic differences between haplogroups in response to elevated temperatures are causally related to mitochondrial sequence differences. However, because the less common B2 haplogroup was insufficiently represented in the previous study (Zeh *et al.* 2012), rigorous assessment of possible mitochondrial haplotype effects requires a more equitable representation of the two haplogroups. Here, I report the results of a new simulated climate warming study in *C. scorpioides* in which the representation of the relatively rare B2 haplotype is greatly increased. My results indicate significant mitochondrial haplogroup effects on survivorship and sperm production in response to elevated temperature, and these findings have important implications for our understanding of the consequences of climate warming for tropical biodiversity.

## **MATERIALS AND METHODS**

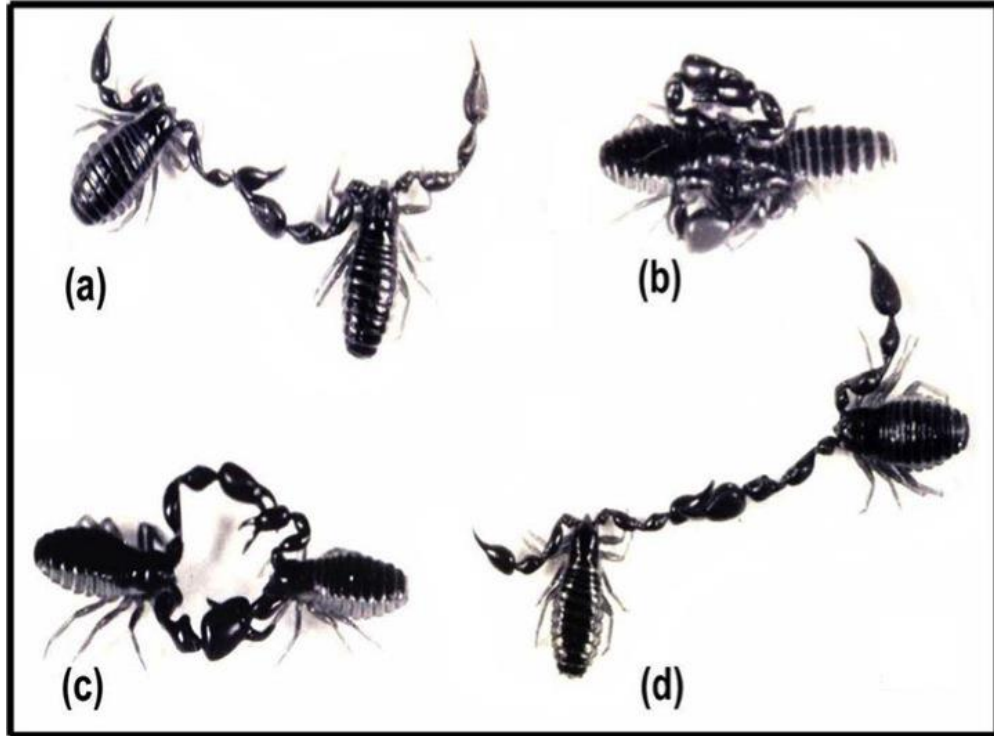
### **The Study Organism**

*Natural history and reproductive biology*

Distributed throughout the rain forests of Central and South America, *C. scorpioides* inhabits decaying trees in the families Moraceae and Apocynaceae (Zeh *et al.*, 1994; 2003), and disperses between habitats on the abdomen of the giant harlequin beetle, *Acrocinus longimanus* (Zeh *et al.*, 1997). The reproductive biology of this pseudoscorpion is ideally suited for carrying out an investigation of the life history, morphological and reproductive consequences of climate warming for a tropical ectotherm. Males transfer sperm to females in discrete packets via a stalked spermatophore deposited on the substrate (Fig. 1). Mating involves a sequence of stereotypical behaviors, in which the male grasps the female and holds her in a stationary position while he constructs and deposits the spermatophore (Fig. 2). Matings can be interrupted immediately following spermatophore deposition, and the sperm packet collected for assessment of sperm quantity and quality (Bonilla *et al.*, 2011). Females are viviparous, and nourish developing embryos in an external, transparent brood sac, overlying the genital aperture (Weygoldt, 1969; Koop *et al.*, 2009). This “external womb” form of viviparity makes possible non-invasive monitoring of female reproductive status and embryological development (Fig. 3; Newcomer *et al.*, 1999).



**Figure 1.** *Cordylocheres scorpioides* spermatophore consisting of a stalk (out of focus) attached to the substrate, a ball of fluid, and, at the apex of the stalk, a sperm packet containing the sperm.



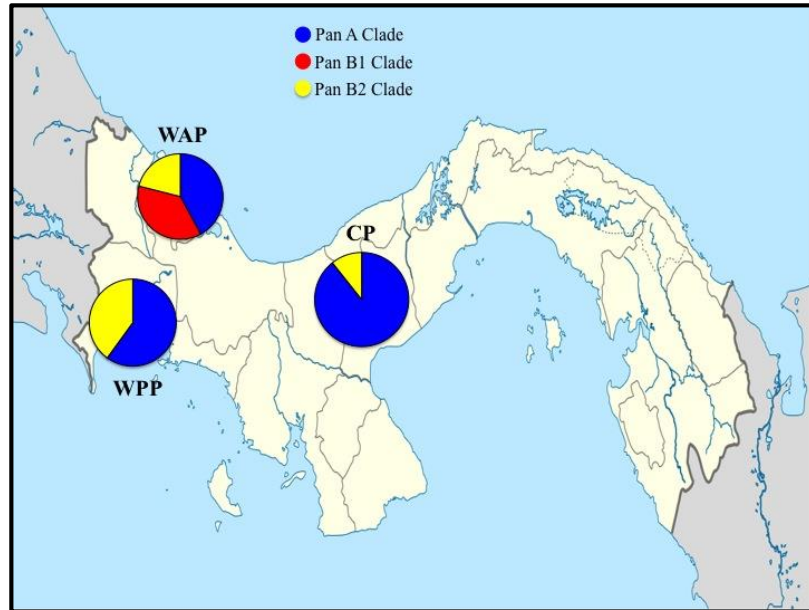
**Figure 2.** Mating sequence in which the male: (a) grasps the female's pedipalp to initiate mating; (b) holds her in a stationary position while he constructs and deposits a spermatophore on the substrate; (c) reverses and attempts to pull the female over the spermatophore, and (d) maintains his hold on the female during the sperm uptake phase.



**Figure 3.** (a) Ventral view of a *C. scorpioides* female carrying a transparent, external brood sac containing early-stage embryos; (b) first-stage nymphs (protonymphs) birthing from the brood sac.

#### *Mitochondrial haplotype variation*

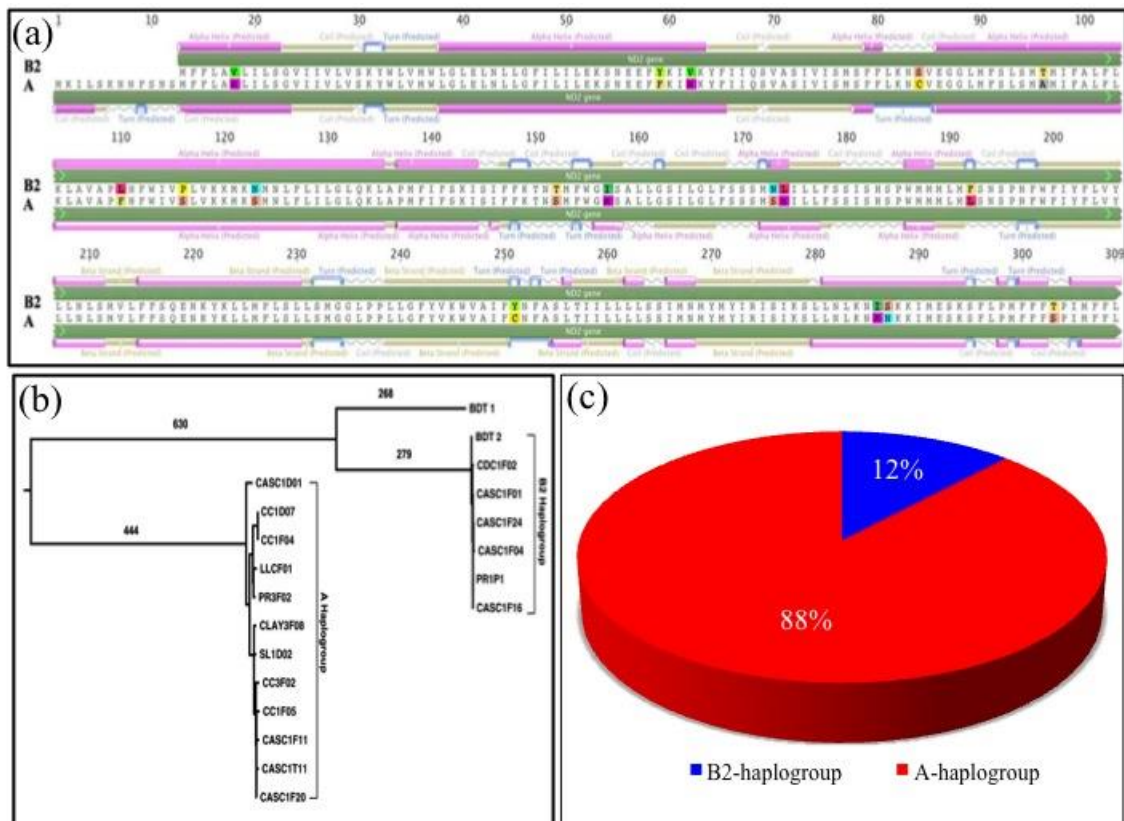
Mitochondrial DNA sequence variation, a potentially important factor influencing levels of nuclear gene expression (Innocenti *et al.*, 2011) and evolutionary response to elevated temperature and increased metabolic demand (Wallace, 2007), is extensive in *C. scorpioides*, with highly divergent haplotypes known to occur sympatrically in western and central Panamá (Fig. 4. Zeh *et al.*, 2003, 2012).



**Figure 4.** Three divergent mitochondrial haplogroups (PAN A, B1 and B2 clades) occur sympatrically in western Panamá. The PAN B2 clade co-occurs at a relatively low frequency of ~12% with the PAN A clade in central Panamá.

The pseudoscorpions used in the simulated climate warming study reported here were drawn from a large laboratory population established from over 350 *C. scorpioides* individuals collected in 2006 and 2008 from six locations spanning a 60-kilometer region of lowland rain forest in central Panamá. Sixty-six of the matriline in this laboratory population were assayed for the ND2 NADH dehydrogenase 2 (ND2) mitochondrial gene sequence. Whole mitochondrial genome sequencing and phylogenetic analysis of 20 of the 66 matriline revealed that the two highly divergent mitochondrial haplogroups, Clades A and B2, coexist in central Panamá (Zeh *et al.* 2012). For the ND2 gene, diversity within the clades was low, with a mean uncorrected nucleotide distance ( $p$ ) of 0.0065 in Clade A, and zero in Clade B2. By contrast, diversity between the two clades

was very high (mean  $p = 0.0756$ ), with up to 18 amino acid differences in the ND2 gene, and approximately 7.5% between-clade divergence, involving more than 1,000 nucleotide substitutions across the entire mitochondrial genome (Fig. 5). Eight of the 66 matriline (12%) were found to carry B2-haplotype mitochondria, while the remaining 58 carry the more common A haplotype. This *C. scorpioides* laboratory population has been maintained for a minimum of 14 generations by mating individuals randomly with respect to mitochondrial haplotype, resulting in mitochondrial clades with a largely homogeneous genetic background. Consequently, any systematic differences between haplogroups in their response to increased temperature are likely to be due to differences in mitochondrial rather than nuclear DNA sequence.



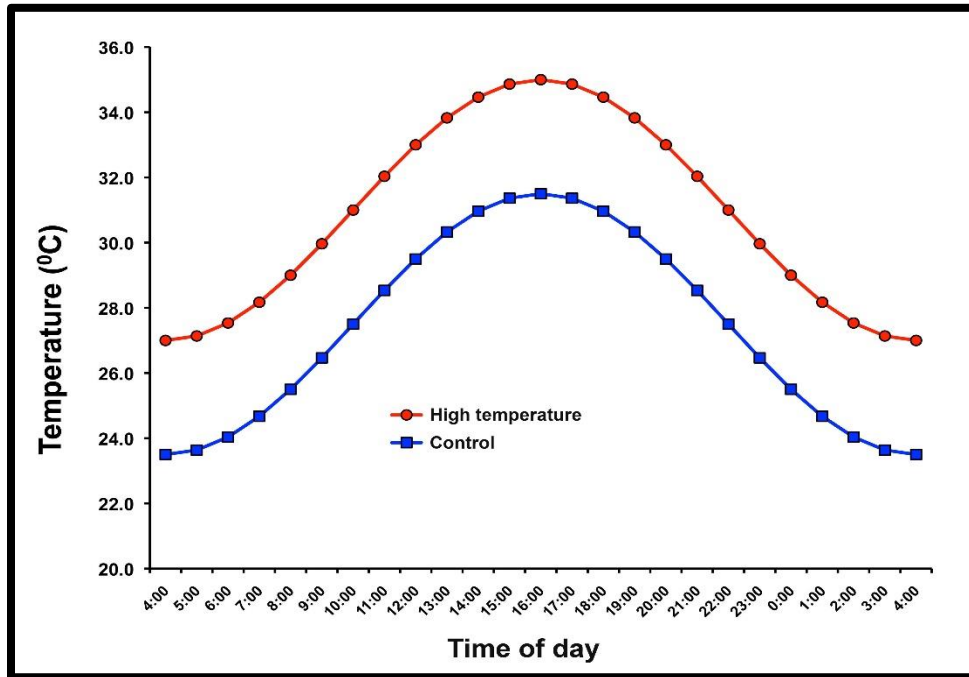


**Figure 5.** Sequencing and phylogenetic analysis of mitochondrial haplotypes. (a) Amino acid differences between Clades A and B2 in the ND2 gene. (b) *Cordylochernes scorpioides* mitochondrial phylogram based on whole-genome sequences of 20 matriline, using maximum parsimony. The number of nucleotide substitutions are shown for main branches. (c) Frequency of mitochondrial haplogroups in the *C. scorpioides* laboratory population.

### **Experimental Design**

To investigate the potential effects of mitochondrial sequence variation on response to increased temperature, the simulated climate warming study (Zeh *et al.* 2012) was repeated, this time increasing the representation of families carrying B2 mitochondria. As in the previous study, virgin females from the laboratory population were each mated to a randomly selected, unrelated male. These females produced 13 full-sibling families carrying B2 haplotype mitochondria and 21 A haplotype families. A split-brood design was used, in which 40 protonymphs (first-stage nymphs) from each female were randomly assigned after birth, 20 to a control treatment (C) and 20 a high temperature treatment (H). The temperature used for the control treatment was determined from average daily shaded air temperature minima and maxima records for the El Claro site on Barro Colorado Island, Panamá collected between 1994 and 2005. This pseudoscorpion most commonly inhabits large fallen Ficus trees which create gaps in the forest that are very comparable to the El Claro site. Climate warming effects were simulated in the high temperature treatment by increasing the temperature 3.5 °C above the control. To mimic environmental conditions experienced by *C. scorpioides* in nature, relative humidity was maintained at 85%, and we used a sine function to vary temperature on a diurnal cycle in

both control and high temperature incubators (Fig. 6). The incubators used for the two temperature treatments were located in a single room and were identical (Percival I-36NL), except for temperature settings.



**Figure 6.** Diurnal temperature profiles for the control (blue) and high temperature (red) treatments.

Nymphs were reared in individual vials to ensure virginity, as described elsewhere (Zeh *et al.* 2005). To determine developmental time from birth to adult, each vial was visually inspected daily in the period between 27 days and 70 days after birth for the presence of an adult. Vials not yielding an adult after 70 days were emptied and inspected, but no nymphs or adults were found, and these individuals were scored as having died during development. Since males and females are morphologically indistinguishable as nymphs, gender was determined only for individuals that survived to the adult stage (N = 963), and the effect of increased temperature on male and female survivorship was assessed,

using a paired comparison by full-sib family of the proportion of survivors that were male in the two treatments.

### **Morphometrics**

To investigate the effect of mitochondrial haplotype on male and female size in the C and H treatments, adults were photographed, held flat under a glass slide with pedipalps fully extended, at high magnification (approximately 30x), using an ALTRA20 digital camera attached to an Olympus SZ6145TR stereomicroscope (Center Valley, PA, USA). For a subset of these images (N = 338), ImageJ 1.43 (National Institutes of Health, Bethesda, MD, USA) was used to measure five cephalothorax and pedipalp traits that become fixed in size at the terminal molt to the adult stage. These five traits were: moveable finger length, chela hand length, chela hand depth, tibia depth, and cephalothorax length (Fig. 7). Principal component analysis of these five morphological traits was used to generate composite measures of size (PC1) and shape (PC2). The trait most tightly correlated with PC1 score was hand depth in males ( $r = 0.97$ ) and cephalothorax length in females ( $r = 0.95$ ). These traits were therefore used as size covariates in analyses of haplotype effects on response to increased temperature.



**Figure 7.** *Cordylochernes scorpioides* male depicting the five measured traits: (1) chela finger length; (2) hand length; (3) hand depth; (4) tibia depth; and (5) cephalothorax length.

### **Male Fertility Traits**

Male fertility traits in the C and H treatments were evaluated for a subset of males (N = 190) by staging matings between experimental males and non-experimental, virgin females, collecting sperm packets and quantifying the number and viability of sperm, as described elsewhere (Bonilla *et al.* 2011). In brief, each male was placed in a 28-mm-

diameter mating arena with a female, and the mating was observed under an Olympus SZ6145TR stereomicroscope. Immediately after spermatophore deposition, the mating was interrupted, and the sperm packet was collected by adhesion to a dissecting needle. Sperm packets were ruptured in 9  $\mu\text{L}$  of phosphate buffered saline (140 mM NaCl; 2.7 mM KCl; 8.1 mM  $\text{Na}_2\text{HPO}_4$ ; 8.1 mM  $\text{KH}_2\text{PO}_4$ ; pH = 7.4) to disperse the sperm. After the addition of 1  $\mu\text{L}$  of a 1:50 dilution of 1 mM SYBR 14, the sperm solution was incubated in the dark for 10 min before adding 1  $\mu\text{L}$  of 2.4 mM propidium iodide (Invitrogen Live/Dead Sperm Viability Kit). The sample was then incubated in the dark for a further 10 min. SYBR 14 is a membrane-permeant, nucleic-acid stain that labels live sperm with green fluorescence, while propidium iodide is membrane-impermeant and labels membrane-compromised (inviable) sperm with red fluorescence. Each 11- $\mu\text{L}$  stained sample was transferred to a hemocytometer, and viewed under a range of magnifications (40X to 400X), using an Olympus BX51 fluorescence microscope equipped with an EM510 dual band pass fluorescence filter cube to enable simultaneous counting of SYBR 14 and propidium iodide stained sperm. The total number of sperm was estimated by multiplying the number of sperm counted in a 0.9- $\mu\text{L}$  volume of the sample by a factor of 12.2 (11  $\mu\text{L}/0.9 \mu\text{L}$ ). Sperm viability was estimated as the number of live sperm divided by the total number of sperm counted.

### **Statistical Analyses**

Data from this study and the previous simulated climate warming study (Zeh *et al.* 2012) were pooled, yielding 51 A-haplogroup families, 16 B2-haplogroup families, and a total

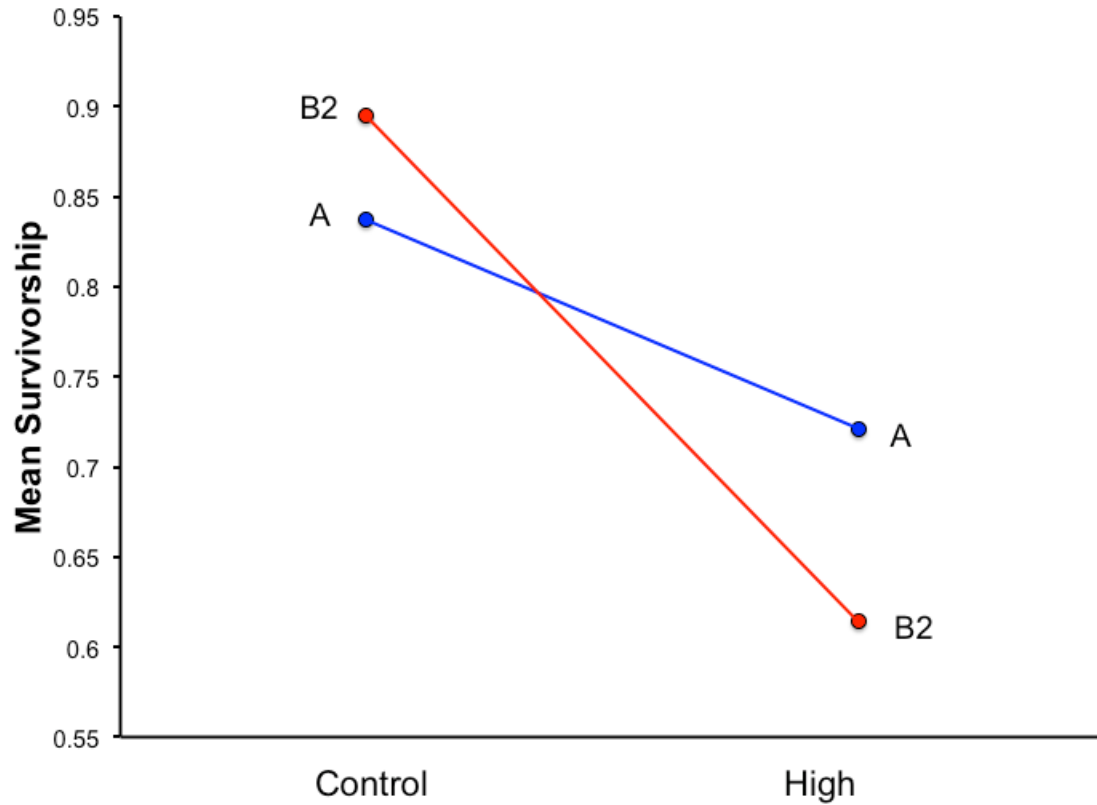
of 1,583 adults for statistical analyses. Temperature and haplotype effects on survival, developmental time, morphology and male reproductive traits were analyzed, using a general linear mixed model (GLMM), as performed in PROC GLIMMIX in SAS, v9.3 (SAS 2011). We avoided pseudoreplication and controlled for genetic and maternal effects by including full-sibling family identity in the models as a random effect. Because morphological traits were normally or approximately normally distributed, GLMMs for analyzing these variables incorporated a Gaussian (Normal) distribution, an identity link function, a Laplace maximum likelihood approximation, and the SAS containment method for determining degrees of freedom. Embryo and protonymph count data were square root transformed and analyzed as above. To accommodate non-normality and overdispersion in the developmental time and sperm number data, GLMMs for analyzing these variables incorporated a log link function, a Gauss-Hermite Quadrature maximum likelihood approximation, and a generalized Poisson mixed model for overdispersed count data (SAS 2011: 3123-3124). Sperm viability was evaluated as the proportion of live sperm in an ejaculate and was not normally distributed. We therefore analyzed this response variable, using the GLIMMIX logit link function to fit a binomial response variable. For all hypothesis testing, we used two-tailed P values.

## **RESULTS**

Consistent with previous studies (Zeh *et al.* 2012, 2014), temperature treatment exerted a significant effect on all of the life history, morphological and reproductive traits assessed in this study.

### **Survivorship and Developmental Rate**

Individuals reared in high temperatures exhibited decreased survivorship compared to their control counterparts ( $P < 0.0001$ ). There was also a significant interaction between haplotype and temperature treatment ( $P = 0.0108$ ). Although the rare B2 haplotype survived slightly but not significantly better than the more common A haplotype when reared at control temperature, the B2 haplotype individuals survived significantly less well than the A haplotype individuals when subjected to high temperatures (Fig. 8; Table 1).



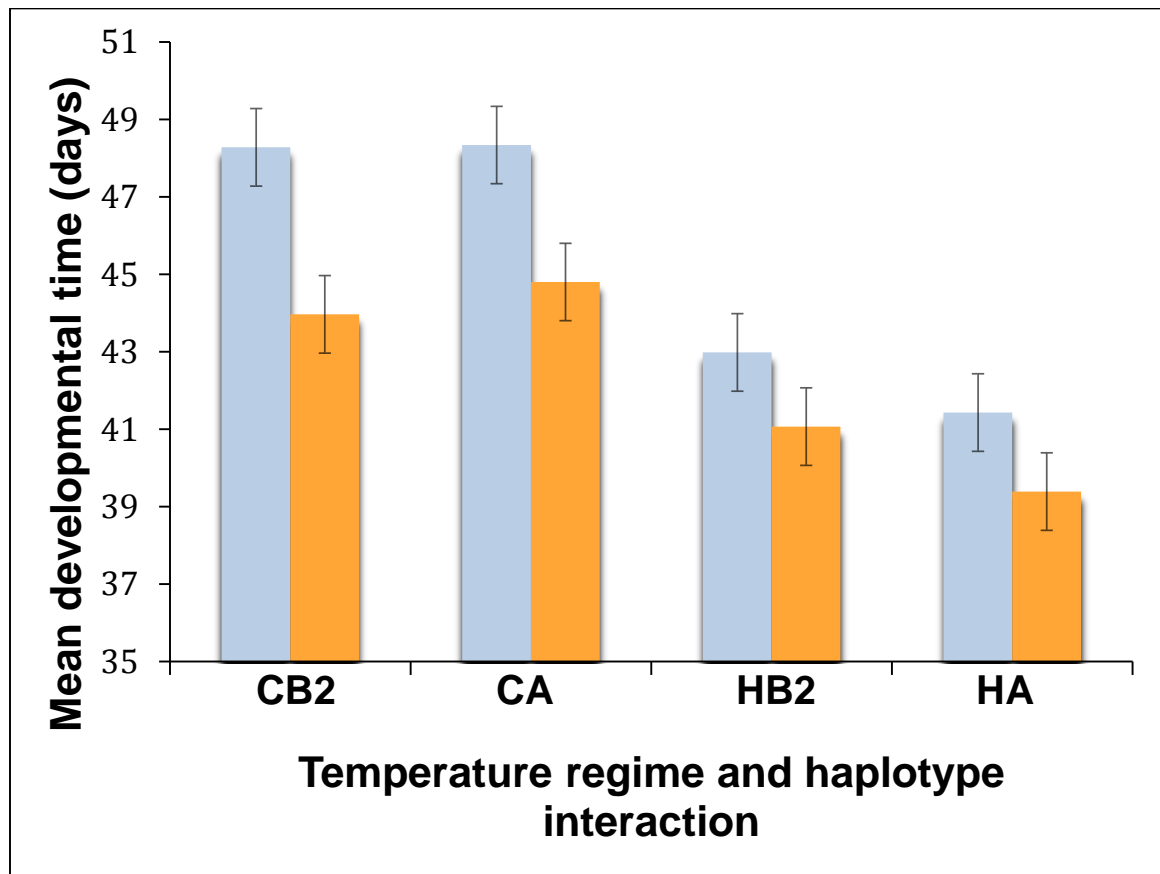
**Figure 8.** Survivorship of the B2 and A haplotype individuals when subjected to control and high temperature treatments.

**Table 1.** Summary of Glimmix analyses for survivorship (proportion of individuals in a full sibling family that survived to the adult size).

N=39	F ratio	P value
<b>Temperature treatment</b>	<b>37.54</b>	<b>&lt; 0.0001</b>
Haplogroup	0.02	0.8899
<b>Temp*Haplo</b>	<b>6.89</b>	<b>0.0108</b>



Reared at the high temperature, both males and females developed much faster than their control counterparts. However, there was no significant interaction between mitochondrial haplotype and temperature treatment for developmental time in either sex (Fig. 9; Table 2).



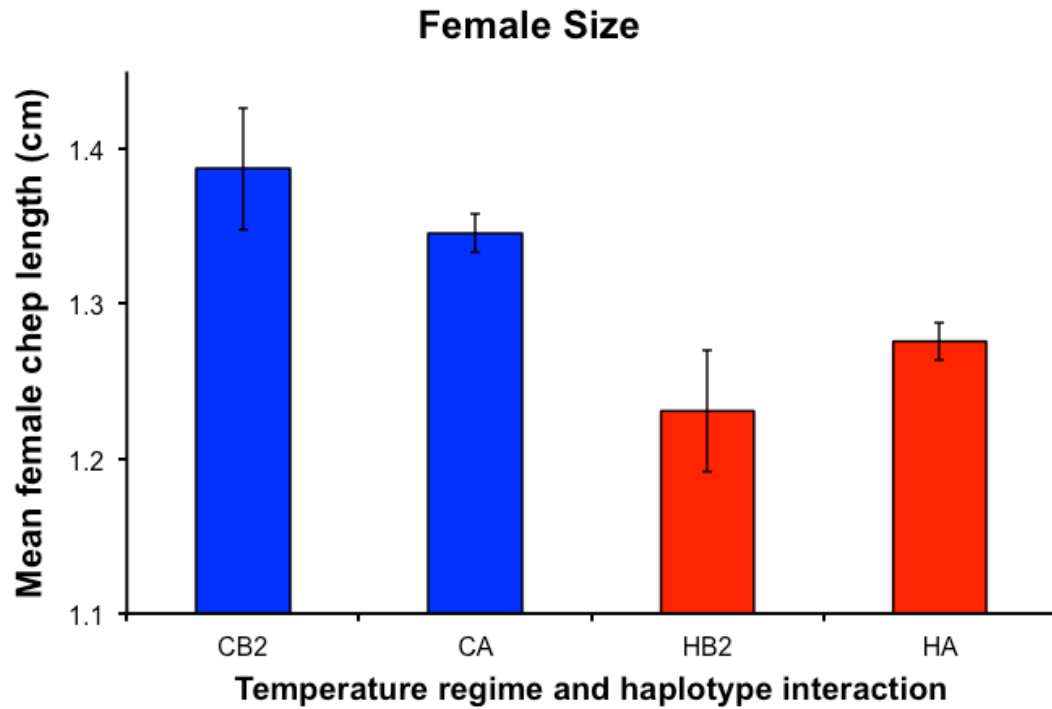
**Figure 9.** Mean ( $\pm$  SE) developmental time in days by haplotype and temperature treatment. Means for males (light blue) and females (orange) were calculated separately for B2 haplotype individuals at the control (CB2) and high temperature (HB2) and A haplotype individuals at the control (CA) and high temperature (HA).

**Table 2.** Summary of GLIMMIX analyses for developmental time.

N=822 FEMALES	F ratio	P value
<b>Temperature treatment</b>	<b>18.43</b>	<b>&lt;0.0001</b>
Haplogroup	0.19	0.6647
Temp*Haplo	1.69	0.1960
N=761 MALES	F ratio	P value
<b>Temperature treatment</b>	<b>39.12</b>	<b>&lt;0.0001</b>
Haplogroup	0.59	0.4449
Temp*Haplo	0.68	0.4095

### **Morphometrics**

In females, variation in overall size is most closely correlated with the sexually monomorphic trait, cephalothorax length (Zeh et al. 2012, 2014) and we therefore used this trait in statistical analysis of female size. Although there was a significant temperature effect of temperature on female cephalothorax length, ( $P < 0.003$ ), the effect of haplotype was not significant ( $P = 0.9595$ ) and there was no significant interaction between mitochondrial haplotype and temperature ( $P = 0.1417$ ) (Fig. 10; Table 3).



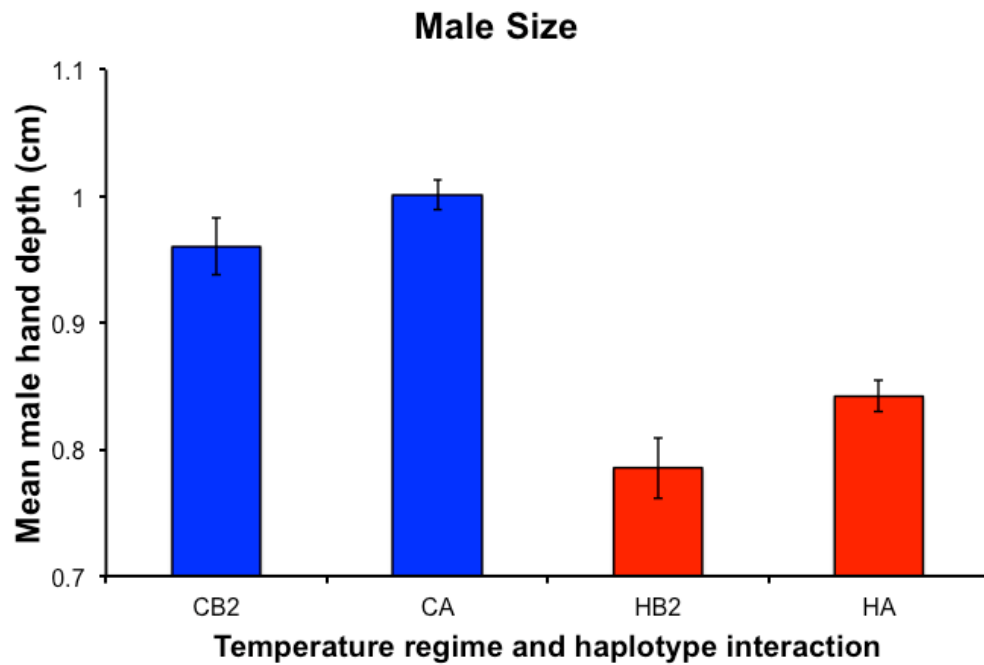
**Figure 10.** Mean female cephalothorax length by haplotype and temperature treatment.

**Table 3.** Summary of GLIMMIX analyses for female size as measured by the sexually monomorphic trait, cephalothorax length.

N= 129	F ratio	P value
<b>Temperature treatment</b>	<b>15.13</b>	<b>&lt;0.003</b>
Haplogroup	0.00	0.9595
Temp*Haplo	2.18	0.1417

Since variation in overall size in males is most closely correlated with the sexually dimorphic trait, chela hand depth (Zeh et al. 2012, 2014), this trait was used for statistical analysis of male size. Temperature exerted a significant effect on male size ( $P < 0.0001$ ),

as did haplotype ( $P = 0.0099$ ). Males carrying the B2 haplotype had significantly smaller hand depths than A haplotype males in both the control and high temperature treatments (Fig. 11; Table 4).



**Figure 11.** Male chela hand depth by haplotype (A and B2) and temperature treatment (C and H).

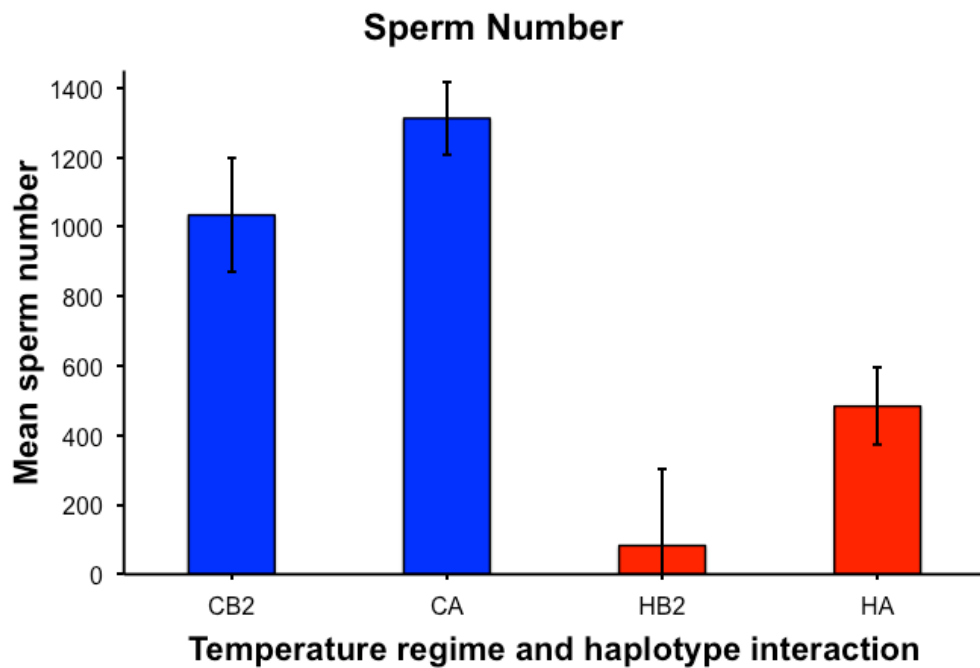
**Table 4.** Summary of GLIMMIX analyses for male size as measured by the sexually dimorphic trait, chela hand depth.

N= 404	F ratio	P value
<b>Temperature treatment</b>	<b>81.56</b>	<b>&lt;0.0001</b>

<b>Haplogroup</b>	<b>6.89</b>	<b>0.0099</b>
Temp*Haplo	0.19	0.6667

### Male Fertility Traits

On average, males subjected to the high temperature treatment produced significantly fewer sperm than did males at the control temperature ( $P < 0.0001$ ). There was also a significant interaction between mitochondrial haplotype and temperature treatment ( $P = 0.0101$ ) for the number of sperm produced (Fig. 12; Table 5).

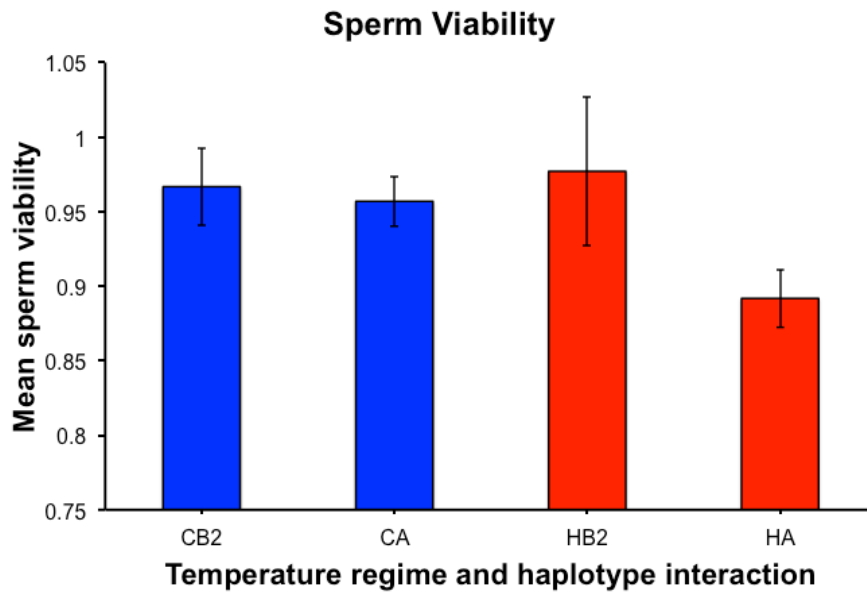


**Figure 12.** Mean sperm number by haplotype (A and B2) and temperature treatment (C and H).

**Table 5.** Summary of GLIMMIX analyses for number of sperm produced.

N= 189	F ratio	P value
<b>Temperature treatment</b>	<b>120.04</b>	<b>&lt;0.0001</b>
Haplogroup	1.63	0.2050
<b>Temp*Haplo</b>	<b>6.89</b>	<b>0.0101</b>

Neither temperature nor mitochondrial haplotype significantly affected sperm viability and the interaction between the two factors was not significant (Figure 13; Table 6).



**Figure 13.** Viability of the collected sperm produced by males.

**Table 6.** Summary of GLIMMIX analyses for sperm viability.

N=189	F ratio	P value
Temperature treatment	0.79	<0.3785
Haplogroup	2.30	0.1339

Temp*Haplo	1.48	0.2271
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## Discussion

Our results indicate that an increase in environmental temperatures is likely to lead to a decrease in genetic diversity. In 2012, the Zeh lab conducted a study that simulated the projected temperature increase for the tropics (IPCC 2007). The results revealed that the projected temperature increase has significant negative impacts on the tropical ectotherm *C. scorpioides*. Since metabolic activity of ectotherms increases exponentially as a result of increasing temperatures, small changes in environmental temperatures have been shown to have great effects on survivorship, developmental time, size, and reproductive success in pseudoscorpions (Zeh *et al.* 2012). Given that the tropics are composed mostly of ectotherms, these findings have major implications for tropical biodiversity. As a follow-up to this study, my research project focused on the influence of mitochondrial genetic variation on *C. scorpioides*' response to elevated temperature, and hence the potential for this tropical ectotherm to adapt to climate warming.

Significant effects of temperature treatment were consistent with previous findings (Zeh *et al.* 2012, 2014). Overall, individuals subjected to the elevated temperature experienced decreased survivorship, faster developmental time, reduced adult size, and decreased reproductive success. A significant interaction was shown between mitochondrial haplotype and temperature treatment in survivorship and total sperm number. The B2 haplogroup proved to be more sensitive to increased temperatures for both traits. B2

males subjected to the high temperature treatment produced significantly fewer sperm and showed a dramatic reduction in survivorship relative to A males. These findings suggest that mitochondrial haplotype influences the response to elevated environmental temperatures. These results add to previous studies that have shown an association of mitochondrial haplotype to variation in environmental adaptation, disease susceptibility, and aging (Kelley *et al.* 2013). Our findings suggest that natural mitochondrial genetic variation may serve as a silver lining to climate warming, as there is the potential for selection to act on this reservoir of genetic variation within populations. Aside from behavioral modifications in response to climate warming, such as migration to higher elevations (Colwell *et al.* 2008), this genetic reservoir can provide an alternative avenue for mitigating the effects of increased temperatures.

Here, we focused on the consequences for *C. scorpioides* of a one-step increase in temperature from the current temperature in this pseudoscorpion's natural habitat to the projected temperature in the tropics by the end of the century. However, temperature change in nature does not occur this abruptly, and future studies should therefore be aimed at investigating the effects of mitochondrial DNA sequence variation in populations subjected to incremental increases in temperature. In addition, still to be determined is the mechanism underlying the greater sensitivity of the B2 haplotype to elevated temperature. The differing mitochondrial haplotype responses reported here could, for example, be due to differences in the production of reactive oxygen species in the mitochondria that damage important cellular proteins and/or negatively impact respiration and metabolism. Nonetheless, it seems clear that level of mitochondrial



haplotype variation in tropical ectotherms, such as *C. scorpioides*, is likely to play an important role in determining the consequences of climate warming for global biodiversity.

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