

**Geographic variation in nesting behavior and thermally-induced offspring phenotypes in a  
widespread reptile**

by

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## DEDICATION

This thesis is dedicated to all the friends and family who have supported me while trying to further my education and understanding of the natural world. I could not have done any of this without their love and support.

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## ABSTRACT

Taxa with large geographic ranges experience considerable variation in climate. To the extent these organisms have environmentally sensitive traits, they provide an excellent opportunity to evaluate how different populations have responded to dissimilar local climates. These eco-evolutionary processes are of particular interest in organisms with traits that are intrinsically connected to temperature, such as embryonic development in oviparous taxa. Maternal effects, particularly oviposition-site choice, greatly affect offspring phenotypes and survival, and thus have the potential to mitigate the effects of divergent climatic conditions across a species range.

In our first study, we delineated natural nesting areas in each of six populations of painted turtles (*Chrysemys picta*) between the Mississippi River and the Pacific Ocean, and quantified spatial and temporal variation in nest microhabitat characteristics, such as thermal environment and canopy cover. To quantify the microhabitats available for females to choose, we also identified sites within the nesting area of each location that were representative of available shade cover. Females nested non-randomly, generally selecting nest sites with less canopy cover and therefore a warmer nesting environment when compared to the area available for nesting in a given location across the wide geographic range. Natural nest microhabitats differed among locations, but did not follow a predictable pattern of varying with latitude or historic mean air temperature during development. The distributions of our thermal data suggest that females are choosing nest sites to buffer against developmental minimum temperatures rather than hot conditions. Thus, nest-site choice may be unlikely to compensate for the novel stressor of rapidly increasing ambient temperatures in these populations.

Lastly, we examined potentially adaptive spatial patterns of phenotypic variation in a widespread vertebrate, we quantified fitness-related embryonic and hatchling traits of the painted turtle (*Chrysemys picta*) from seven locations across its geographic range (in Idaho, Minnesota, Oregon, Illinois, Nebraska, Kansas, and New Mexico). We incubated eggs from these sites under constant conditions across a range of environmentally relevant temperatures. Thermal reaction norms for developmental rate, hatchling mass, carapace width, and survival to hatching varied among localities. We further found evidence to support local adaptation in some of these traits in common-garden conditions. However, latitude and the 30-year mean July air temperature (i.e., during the middle of development) for each location were not strong predictors of these geographic differences. These findings suggest that common proxies, such as latitude, used to encapsulate geographic patterns in phenotypic variation may not be reliable predictors. Thus, complex interactions between abiotic and biotic factors likely drive among-population phenotypic variation. Understanding spatial variation in key traits provides important perspective on current adaptation to climatic conditions and future phenotypic responses to projected climate change.

Overall, these studies have contributed to the existing body of literature leverage an integrative approach that uses lab and field studies to assist in understanding how these organisms accommodate different thermal conditions during development. Elucidating how organisms with temperature-sensitive traits are persisting in these vastly different environments is key to predicting how they may respond to rapidly changing thermal conditions predicted under climate change models.

## CHAPTER 1 INTRODUCTION: THESIS ORGANIZATION

Adaptation and phenotypic plasticity permit organisms to persist in changing environments (Gienapp *et al.* 2008; Reed *et al.* 2010). Local adaptation occurs when different alleles underpinning different phenotypes are favored in response to phenotypic selection induced by biotic and abiotic factors. Where environmental heterogeneity arises across a species' range, local adaptation can generate geographic variation in phenotypes over a number of generations (Kawecki & Ebert 2004). Phenotypic plasticity, on the other hand, can occur within a generation, producing phenotypes to match local environments without underlying genetic change (Steinger, Roy & Stanton 2003; Ghalambor *et al.* 2007a). In many natural systems, both plastic and genetic variation are factors in forming phenotypes that best match local conditions (Conover & Schultz 1995; Conover, Duffy & Hice 2009).

Species with environmentally-sensitive traits and large geographic ranges make excellent study systems for answering questions about local adaptation and/or phenotypic plasticity (Conover & Schultz 1995; Angilletta 2009; Kingsolver 2009). Specifically, species with broad geographic distributions are comprised of populations that experience different climates and, thus, are expected to match phenotypes to local environmental conditions. In so doing, patterns of intraspecific phenotypic and genetic variation among populations can shed light on microevolutionary potential in response to environmental change.

Of major contemporary concern are the biological impacts of climate warming, as a 1.5 °C global increase of surface temperatures is conservatively predicted by the end of the century (IPCC 2014). An appropriate system to address questions associated with adaptation and biotic responses to climate change, particularly involving temperature, requires three critical

characteristics: (1) The trait of interest is directly impacted by temperature, (2) The temperature-sensitive trait is fundamentally tied to key processes associated with fitness, (3) The system of interest experiences extensive variation in temperatures, spatially and temporally, in a contemporary context.

Maternal effects are important to consider when addressing questions of ecological and evolutionary interest. Maternal effects are non-genetic factors, such as the maternal environment or phenotype, that influence offspring phenotypes outside of their own genotype or the environment they experience (Bernardo 1996). The ecological and evolutionary consequences of maternal effects as factors influencing phenotypic patterns and dynamics are complex (Kirkpatrick & Lande 1989), yet maternal effects occur in most taxa (Mousseau & Fox 1998). Maternal effects can influence offspring in a variety of ways, including but not limited to: maternal nutrition, transmission of pathogens or antibodies, propagule size, and/or oviposition-site choice (Mather & Jinks 1971; Bernardo 1996; Refsnider & Janzen 2010). Oviposition, or nest-site choice, is a critical life-history trait in oviparous organisms that lack parental care, and has repercussions for female and offspring fitness (Resetarits 1996). Through nest-site choice, females can influence offspring survival and phenotype by selecting specific microhabitats for nests (Petranka & Petranka 1981; Bernardo 1996; Liebezeit & George 2002; Spence, Ashton & Smith 2007; Refsnider & Janzen 2010; Mitchell, Warner & Janzen 2013; Mitchell, Maciel & Janzen 2015; Refsnider 2016). Thus, such maternal effects can play a central role in both adaptive and plastic responses to selection induced by local abiotic and biotic factors.

Using the model system of a widespread North American ectothermic vertebrate (the painted turtle, *Chrysemys picta*), we evaluated how disparate populations accommodate geographic variation in climate, specifically with regards to the temperatures they experience during

development. *Chrysemys picta* is an appropriate model system for understanding plasticity and local adaptation: (1) Temperature affects multiple fitness-related offspring phenotypes in *C. picta*, such as sex (Bull & Vogt 1979), morphological abnormalities (Telemeco *et al.* 2013), body size (Janzen & Morjan 2002), and developmental rate (Gutzke *et al.* 1987). (2) Many of these temperature-sensitive phenotypes are related to organismal fitness and population demographics. For example, high incubation temperatures induce morphological abnormalities and, in nature, these abnormalities are present more frequently in neonates than in adult *C. picta*, suggesting that hatchlings with abnormalities are less likely to survive to adulthood (Telemeco *et al.* 2013). Of note, nest-site choice can alter the thermal environment of the nest (Janzen 1994; Mitchell, Maciel & Janzen 2013; Refsnider *et al.* 2014; Mitchell, Maciel & Janzen 2015). This behavior appears to be plastic in nature but has contextual heritability (McGaugh *et al.* 2009; Refsnider & Janzen 2012), therefore the phenotypic framework for selection to act upon (along with heritable underpinnings) is potentially present in this system. (3) *Chrysemys picta* is a geographically widespread species, ranging from the east to west coasts of the continental United States and from Mexico to Canada (Starkey *et al.* 2003; Ernst & Lovich 2009).

The goal of this thesis was to elucidate how maternal and environmental effects during development, which may differ among populations, influence offspring phenotypes in a geographically widespread ectotherm. In the first data chapter, I examined the maternal effect of nest-site choice by characterizing nest microhabitats. Quantifying variation in nest microhabitats took place at a large geographic scale, and across multiple nesting seasons. I also compared microhabitat characteristics of natural nest sites to the microhabitat characteristics available in a given location for the females to choose from. In the second data chapter, I conducted a common-garden experiment, incubating *C. picta* eggs from geographically disparate regions

across a range of viable constant temperatures to measure phenotypic variation potentially associated with the developmental thermal environment and location of origin.

These contributions to the existing body of literature leverage an integrative approach that uses lab and field studies to assist in understanding how these organisms accommodate different thermal conditions during development. Elucidating how organisms with temperature-sensitive traits – and which are unlikely to migrate to climatically different macrohabitats without anthropogenic assistance (Mitchell & Janzen 2010) – are persisting in these vastly different environments is key to predicting how they may respond to rapidly changing thermal conditions predicted under climate change models.

## **Thesis Organization**

### **Chapter 1: Mother knows best: nest-site choice homogenizes embryo thermal environments among populations in a widespread turtle**

Taxa with large geographic ranges experience considerable variation in climate. To the extent these organisms have environmentally sensitive traits, they provide an excellent opportunity to evaluate how different populations have responded to dissimilar local climates. Maternal effects, particularly oviposition-site choice, greatly affect offspring phenotypes and survival, and thus have the potential to mitigate the effects of divergent climatic conditions across a species range. We delineated natural nesting areas in each of six populations of painted turtles (*Chrysemys picta*) between the Mississippi River and the Pacific Ocean, and quantified spatial and temporal

variation in nest microhabitat characteristics, such as thermal environment and canopy cover. To quantify the microhabitats available for females to choose, we also identified sites within the nesting area of each location that were representative of available shade cover. Females nested non-randomly, generally selecting nest sites with less canopy cover and therefore a warmer nesting environment when compared to the area available for nesting in a given location across the wide geographic range. Natural nest microhabitats differed among locations, but did not follow a predictable pattern of varying with latitude or historic mean air temperature during development. The distributions of our thermal data suggest that females are choosing nest sites to buffer against developmental minimum temperatures rather than hot conditions. Thus, nest-site choice may be unlikely to compensate for the novel stressor of rapidly increasing ambient temperatures in these populations.

## **Chapter 2: Geographic variation in thermal sensitivity of early life traits in a widespread reptile**

Organisms with large geographic distributions experience a great diversity of climatic conditions, requiring local adaptation and/or phenotypic plasticity. These eco-evolutionary processes are of particular interest in organisms with traits that are intrinsically connected to temperature, such as embryonic development in oviparous taxa. To examine potentially adaptive spatial patterns of phenotypic variation in a widespread vertebrate, we quantified fitness-related embryonic and hatchling traits of the painted turtle (*Chrysemys picta*) from seven locations across its geographic range (in Idaho, Minnesota, Oregon, Illinois, Nebraska, Kansas, and New Mexico). We incubated eggs from these sites under constant conditions across a range of environmentally relevant temperatures. Thermal reaction norms for developmental rate,

hatchling mass, carapace width, and survival to hatching varied among localities. We further found evidence to support local adaptation in some of these traits in common-garden conditions. However, latitude and the 30-year mean July air temperature (i.e., during the middle of development) for each location were not strong predictors of these geographic differences. These findings suggest that common proxies, such as latitude, used to encapsulate geographic patterns in phenotypic variation may not be reliable predictors. Thus, complex interactions between abiotic and biotic factors likely drive among-population phenotypic variation. Understanding spatial variation in key traits provides important perspective on current adaptation to climatic conditions and future phenotypic responses to projected climate change.

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## CHAPTER 2

MOTHER KNOWS BEST: NEST-SITE CHOICE HOMOGENIZED EMBRYO THERMAL  
ENVIRONMENTS AMONG POPULATIONS IN A WIDESPREAD TURTLE

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**ABSTRACT**

Taxa with large geographic ranges experience considerable variation in climate. To the extent these organisms have environmentally sensitive traits, they provide an excellent opportunity to evaluate how different populations have responded to dissimilar local climates. Maternal effects, particularly oviposition-site choice, greatly affect offspring phenotypes and survival, and thus have the potential to mitigate the effects of divergent climatic conditions across a species range. We delineated natural nesting areas in each of six populations of painted turtles (*Chrysemys picta*) between the Mississippi River and the Pacific Ocean, and quantified spatial and temporal variation in nest microhabitat characteristics, such as thermal environment and canopy cover. To quantify the microhabitats available for females to choose, we also identified sites within the nesting area of each location that were representative of available shade cover. Females nested non-randomly, generally selecting nest sites with less canopy cover and therefore a warmer nesting environment when compared to the area available for nesting in a given location across the wide geographic range. Natural nest microhabitats differed among locations, but did not follow a predictable pattern of varying with latitude or historic mean air temperature during development. The distributions of our thermal data suggest that females are choosing nest sites to buffer against developmental minimum temperatures rather than hot conditions. Thus, nest-site choice may be unlikely to compensate for the novel stressor of rapidly increasing global temperatures in these populations.

## INTRODUCTION

Species with environmentally sensitive traits and large geographic ranges make excellent study systems for answering questions about local adaptation and/or phenotypic plasticity (Conover & Schultz 1995; Angilletta 2009; Kingsolver 2009). Adaptation and phenotypic plasticity permit organisms to persist in changing environments (Gienapp *et al.* 2008; Reed *et al.* 2010). Of major contemporary concern are the biological impacts of climate warming, as a 1.5 C° global increase of surface temperatures is conservatively predicted by the end of the century (IPCC 2014). An appropriate system to address questions associated with adaptation and biotic responses to climate change, particularly temperature, requires three critical characteristics: (1) The trait of interest is directly impacted by temperature, (2) The temperature-sensitive trait is fundamentally tied to critical processes associated with fitness, (3) The system of interest experiences extensive variation in temperatures in a contemporary context.

Beyond the direct impacts of genetics and the environment, non-genetic or epigenetic effects can influence an individual's phenotype. These effects, in turn, are determined by the phenotype and/or the environment experienced by its mother, which are defined as maternal effects (Bernardo 1996; Mousseau & Fox 1998). Maternal effects are ubiquitous, as demonstrated by empirical support across taxa including but not limited to: plants (Roach & Wulff 1987), insects (Mousseau & Dingle 1991), fish (Reznick 1991), amphibians (Forester 1979), birds (Blount *et al.* 2002), reptiles (Mitchell, Maciel & Janzen 2015), and mammals (Cowley 1991). Maternal effects have increasingly been acknowledged as factors that provide individual phenotypic variation as a target for natural selection and even the substrate to drive evolutionary change (Kirkpatrick & Lande 1989; Mousseau & Fox 1998). Even in species that lack parental care, maternal effects have a substantial impact on offspring. For example, when and where a mother

places her offspring is frequently the most influential determinant of offspring success (Bernardo 1996). Indeed, oviposition-site choice is a maternal effect by which females can affect the survival and phenotype of their offspring (Mitchell 1975; Howard 1978; Li & Martin 1991; Shine & Harlow 1996; Refsnider & Janzen 2010; Mitchell *et al.* 2015).

The painted turtle, *Chrysemys picta*, has many characteristics conducive to investigating biotic responses to changing thermal environments and how maternal effects could mitigate the impacts of these changes. First, incubation temperature affects multiple hatchling phenotypes (e.g., body size and growth) (Deeming & Ferguson 1991; Cagle *et al.* 1993; Rhen & Lang 1999; Weisrock & Janzen 1999). Many of these temperature-sensitive phenotypes are related to organismal fitness and population demographics (Morjan 2003a; Freedberg & Taylor 2007). Most notably, *C. picta* exhibits temperature-dependent sex determination (TSD), such that females are produced at warmer incubation temperatures and males at cooler ones (Morjan 2003a). Theory suggests that species with TSD could impact temperature-sensitive traits of their embryos by making temporal or spatial shifts in nesting behavior (Morjan 2003a; Morjan 2003c; Refsnider & Janzen 2010; Refsnider 2016). Finally, *C. picta* is a geographically widespread species, ranging from the east to west coasts of the continental United States and from Mexico to Canada (Starkey *et al.* 2003; Ernst & Lovich 2009). Thus, populations in different parts of the range experience considerably dissimilar local climatic conditions.

Maternal effects contribute to population-level responses to environmental temperatures in turtles. Nest-site choice can influence temperatures experienced by the developing embryos and therefore offspring phenotypes (Janzen & Morjan 2002; Schwanz *et al.* 2009; Schwanz *et al.* 2010). In multiple turtle species, documented repeatability of nest-site choice (e.g., shade cover) provides a behavioral phenotype upon which selection can act (Janzen & Morjan 2002; Kamel &

Mrosovsky 2005). Additionally, nest-site choice in nature associated with a specific microhabitat is heritable following warm winters ( $h^2= 0.19$  in *C. picta*; (McGaugh *et al.* 2009)), providing a genetic component for this behavior to potentially evolve in response to selection. The focus of the present study was thus to examine nest-site choice by characterizing nest microhabitats at both local and broad geographic scales and through time:

(1) How do natural nests differ from potential nest sites (i.e.-representative sites (see methods)) in each study location? We hypothesized that natural nests would have different microhabitat characteristics when compared to the representative sites at each location, predicting that natural nests would have less canopy cover and be warmer than the representative sites (Schwarzkopf & Brooks 1987).

(2) How do natural nests differ among study locations across a wide geographic range? We hypothesized that females would choose nest sites based on different criteria to accommodate temperature differences found across locations. For example, we predicted that mothers from more northern populations would choose nest sites with less canopy cover to make a warmer nest microhabitat because the growing season is shorter. Investigating the effects of the maternal behavior of nest-site choice on microhabitat conditions across geographically widespread populations could generate insights into how this species maintains successful populations in such varied contemporary environments as well as potentially future global increases in temperature.

## **STUDY ORGANISM AND FIELD SITES**

*Study Organism* —We conducted this comparative study using painted turtle (*Chrysemys picta*) populations west of the Mississippi River (Starkey *et al.* 2003; Ernst & Lovich 2009). Females

emerge from freshwater habitats in May and June to excavate shallow, Erlenmeyer flask shaped, subterranean nests with a mean depth of 10 cm in these populations. The painted turtle exhibits type Ia TSD, with females produced at constant incubation temperatures above 29°C and males produced at constant incubation temperatures below 27°C (Ewert, Jackson & Nelson 1994). The temperature-sensitive period corresponds with the middle third of development, which approximately matches with July in our study populations (Janzen 1994).

We studied the nesting biology of *C. picta* at six field sites: Idaho, Illinois, Minnesota, Nebraska, New Mexico, and Oregon (Table 1). These locations range across nearly 33 degrees of longitude from the Mississippi River west to the Pacific coast and across nearly 15 degrees of latitude from the southern range limit near Mexico almost to the Canadian border. Three of these locations (Illinois, Nebraska, and New Mexico) are the foci of previous long-term research studies (Iverson & Smith 1993; Janzen 1994; Morjan 2003b).

## **METHODS AND MATERIALS**

*Natural Nests*— Experienced personnel surveyed nesting activities at each location hourly from sunrise to sunset from May through June. Nesting turtles were monitored from a distance until nest construction was completed. Upon completion, nests were carefully excavated, and a Thermocron iButton programmed to record temperature hourly was wrapped in latex and Parafilm (to provide waterproofing) and placed in the middle of the cavity amongst the eggs. Once the eggs were replaced, in the order they were removed, and the thermal logger was in place, the cavity was backfilled with soil and protected from predation (except at Illinois to avoid interference with ongoing long-term data collection) with 1-cm wire hardware cloth (~25 cm X

25 cm) and mapped for relocation. To quantify shade cover and solar radiance above each nest, a hemispherical photo was taken using a 180° fisheye lens. The camera was placed on a wooden block facing North, directly on top of the nest cavity. These hemiphotos were analyzed using Gap Light Analyzer (Frazer, Canham & Lertzman 1999; Doody *et al.* 2006b). Weather data and magnetic declination were site- and year-specific spanning the month of July ([www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)).

*Representative sites*— Using GIS methodology to create a probability-based spatial sampling design from known canopy cover, 40 generated coordinates within the perimeter of each study location were chosen as a representation of the available nest site canopy structure (Krivoruchko & Butler 2013). After nesting began at each location, Thermocron iButtons were placed at the mean nest depth (10 cm) in these populations (unpublished data). As with the natural nests, a hemispherical photo also was taken at each representative site.

The middle third of incubation corresponds to the temperature-sensitive period where sex is determined irreversibly by incubation temperatures (Bull & Vogt 1981) and is the primary period of overall organogenesis (Cordero & Janzen 2014). Thus, hourly nest and representative site temperatures during this period were converted to constant temperature equivalents (CTEs), which facilitates statistical comparisons (Georges, Limpus & Stoutjesdijk 1994; Georges *et al.* 2005; Telemeco, Abbott & Janzen 2013). The CTE model translates fluctuating temperatures into values comparable to a constant temperature during incubation, calculating the temperature associated with the median developmental rate in *C. picta*.

*Statistical Methods:*

All statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC). A generalized linear model was used to compare temperature and canopy cover across locations and between nest sites and representative sites within locations. Both year nested within location and location were treated as fixed effects. Hemispherical photos were analyzed using a generalized linear model in the same manner. The same photos were used for the representative sites to measure shade cover across all years in this study, but with weather data corresponding to the appropriate nesting season. Representative site photos were analyzed using the same threshold pixel settings as the natural nests to ensure shade cover comparability between years. Percentage canopy openness was arc sin transformed and a Tukey correction was used for multiple comparisons (Sokal & Rohlf 1981).

**RESULTS***Natural Nests Vs Representative Sites*

Data collection occurred from 2012-2016 across 6 localities, with 1821 hemispherical photos taken and 999 thermal traces recorded. Examples of percent canopy cover from specific sites ranged from 20.6% in one nest in Illinois to 97.5% in one nest in Nebraska for the natural nests, and 9.6% in Idaho to 98.4% in New Mexico for the representative sites. In all locations except Nebraska, natural nests had less canopy cover when compared to representative sites (Fig. 1). The percent canopy cover in Nebraska did not differ between the nest sites and representative sites, exhibiting a pattern (opposite) where representative sites had less canopy cover than nests. The percent canopy open differed between site types (natural nests vs. representative sites; ( $F_{1,1810}=206.59$ ,  $p<0.0001$ )) and a type by location interaction also was evident ( $F_{5,1810}=37.81$ ,

$p < 0.0001$ ). The interaction of location and type indicated that the two types of sites differed in percent canopy cover values across locations (Fig. 2). The largest difference between the canopy cover of a location's natural nests and representative sites was 25.8% in Idaho, whereas the smallest difference was 1.4% in Nebraska.

The minimum and maximum raw thermal data (i.e.- single hourly observations) were 3.50-54.50°C for the representative sites and 5.00-54.50°C for the natural nests. The distribution of the CTEs illustrates that females chose nest sites that were warmer than representative sites (Fig. 3). Indeed, the CTEs of natural nests averaged more than 4°C warmer than representative sites (26.2 vs. 21.9 °C;  $F_{1,998} = 325.05$   $p < 0.0001$ ), though the range of temperatures only differed slightly between the nest types (Fig. 4; Table 2). Constant temperature equivalents also differed by location ( $F_{5,998} = 140.89$ ,  $p < 0.0001$ ), location-specific year ( $F_{14,998} = 8.86$ ,  $p < 0.0001$ ), and the interaction between location and type ( $F_{5,998} = 88.41$ ,  $p < 0.0001$ ). Within a location, natural nests were warmer than their representative counterparts, but the differences in CTEs between natural nests and representative sites differed among locations (Fig. 5). The CTEs between Idaho nests and representative sites differed by 8.5°C, whereas in Illinois that difference was only 1.7°C. The differences within a location between the natural nest and representative site CTEs followed a latitudinal pattern in which the higher latitude populations generally had a larger difference between the two values (Fig. 6).

#### *Natural Nests Across Locations*

Canopy cover over nests differed among locations ( $F_{5,1810} = 147.04$ ,  $p < 0.0001$ ), but did not follow a latitudinal pattern. The least squares means overlapped in percent canopy cover for Oregon and Nebraska and for New Mexico and Minnesota (Fig. 7). The CTEs also differed among locations ( $F_{5,376} = 21.27$ ,  $p < 0.0001$ ) and by location-specific year ( $F_{12,376} = 10.54$ ,  $p < 0.0001$ ; Table 3).

Comparisons across years within a location illustrate a complex temporal pattern in which year only occasionally played a major role in influencing nest CTEs (Fig. 8; Table 4).

## DISCUSSION

Our objectives were to evaluate nest microhabitat characteristics of *C. picta* to answer two questions: (1) How do natural nests differ from potential nest sites (i.e. - representative sites) at locations across a wide geographic range? (2) How do natural nests differ among locations across a wide geographic range? With information regarding these questions, we have the potential to elucidate how these organisms with temperature-sensitive traits are persisting in these contemporarily diverse climates. Understanding current organismal strategies for dealing with a wide range of thermal conditions could aid our ability to predict population response to current climate change. We found that nest-site choice by painted turtles may mitigate the impacts of divergent ambient temperatures among populations on developing eggs, such that expectations of nest thermal environments based on latitude and local climate were buffered by nesting behavior (Fig. 5).

The microhabitats – measures of canopy cover and nest thermal environments – between the natural nests and representative sites differed across study locations. The canopy was more open in female-selected nest sites, and therefore these sites were generally warmer, when compared to what was available in a given location overall. Only in Nebraska did canopy cover not differ substantively between natural nests and representative sites. Other potential microhabitat measures that could affect nest conditions experienced by developing embryos that were not examined in this study include substrate type (Farouki 1986; Garmestani *et al.* 2000), slope of the nest site (Schwarzkopf & Brooks 1987; Wood, Bjorndal & Ross 2000), and nest depth

(Refsnider *et al.* 2013). Additionally, a maternal behavior such as nesting phenology (i.e.- when during the nesting season a female oviposits her eggs) could impact the microhabitat experienced by developing embryos (Schwanz & Janzen 2008).

The distributions of the thermal data of natural nests and representative sites indicate that, historically, females appear to have chosen nest sites to buffer thermal minima during development rather than maxima, as hypothesized by (Schwarzkopf & Brooks 1987; Telemeco *et al.* 2017). The developmental thermal minimum is the temperature below which no significant development can take place, which also is true for temperatures above the critical thermal maximum. In *C. picta*, constant incubation temperatures of 14°C and 34°C are the developmental thermal minimum and maximum, respectively (Telemeco, Abbott & Janzen 2013). No natural nest CTEs were outside the thermal minimum or maximum for development (20.1-30.4°C). Some representative sites had CTEs that reached the developmental thermal minimum (14.4-29.4°C), though, and the warmest CTE was cooler than the warmest natural nest CTE. The differences between natural nest and representative site minimum temperatures was notably ~6 °C, with the representative sites being cooler, indicating females may be enhancing nest temperatures.

We detected differences in shade cover and nest thermal environments across study locations. Comparing among locations, not one population consistently experienced the warmest or coolest nest temperatures (CTEs) across years, indicating the importance of temporal variance in thermal conditions. The differences in natural nest microhabitats did not follow a predictable pattern (e.g., latitude or air temperatures), in contrast to findings from other studies of geographic variation in nest-site choice in reptiles (Ewert, Lang & Nelson 2005; Doody *et al.* 2006a). The contrasts among the findings of these studies could be attributable to differences in

methodologies or different species used. Various species potentially have different constraints on development and therefore pressures on maternal behaviors. Interestingly, the aforementioned studies both examined species (common snapping turtle and water dragon) that have type II TSD, whereby females are produced at warm and cool incubation temperatures, and males are produced at intermediate incubation temperatures. Regardless, for painted turtles we uncovered a latitudinal pattern of differences between the mean nest and representative site CTEs: higher latitude locations generally had larger differences between these mean CTEs compared to more southern populations. This pattern suggests females in more northern populations are selecting nest sites to compensate for cooler temperatures of shorter growing seasons at higher latitudes.

Nest-site choice in oviparous reptiles is a maternal effect that significantly influences conditions experienced during development, and has the potential to influence offspring phenotype and survival, including in *C. picta* (Weisrock & Janzen 1999; Mitchell, Maciel & Janzen 2013; Mitchell, Warner & Janzen 2013; Bodensteiner *et al.* 2015; Mitchell *et al.* 2015; Refsnider 2016). Individual females show repeatability in nesting behavior, with certain females consistently choosing specific shade cover year-to-year (Janzen & Morjan 2001). Although there is repeatability in this behavior, the field heritability is relatively low and context dependent (McGaugh *et al.* 2009). The mechanism for how females are choosing suitable nest sites is largely unknown, but has been hypothesized to be a visual cue where females use the overstory canopy cover, instead of immediate thermal cues, to identify appropriate nest sites (Janzen 1994; Morjan & Valenzuela 2001). There is little evidence to support the hypothesis that olfactory cues are used in nest-site choice (Iverson *et al.* 2016). Regardless, if cues for identifying suitable nest sites were to become unstable predictors or mismatched to the environment in the context of climate change, the potential for adverse fitness consequences is considerable.

Oviposition-site choice is a critical life-history trait with the potential to affect maternal fitness and survival, embryo phenotypes and survival, and juvenile performance across oviparous taxa (Resetarits 1996). Proposed hypotheses for nonrandom oviposition-site choice include: maximizing offspring fitness, maximizing maternal survival, modifying offspring phenotypes, proximity to suitable habitat for offspring, maintaining natal philopatry, and indirect oviposition-site choice via mate choice (Refsnider & Janzen 2010). Thus, the ultimate reasons for maintaining oviposition-site choice vary among taxa (and potentially among populations), and are not necessarily mutually exclusive. Regardless, oviposition site-choice remains an important mechanism by which organisms could mitigate the effects of changing environmental conditions. Of concern is the rate at which global climate is currently changing and predicted to continue in the next century (IPCC 2014). The distributions of the thermal data of the natural nests and representative sites indicated that, historically, females have chosen nest sites to buffer thermal minima during development rather than maxima. In one lizard species, if an embryo has a single experience with the developmental thermal minimum, survival can be reduced to ~50% (Levy *et al.* 2015). Although nest-site choice mainly is a behaviorally plastic characteristic in *C. picta* (Refsnider & Janzen 2012), for plasticity to aid in population persistence during periods of environmental change, the future conditions should be within the range of the original conditions that selected for the current reaction norm (Ghalambor *et al.* 2007b; Merilä & Hendry 2014). Thus, based on our findings, it appears plasticity in nesting behavior for buffering developmental thermal maxima has not been under selection, as opposed to selection to supersede developmental thermal minima. Therefore, the rate of global climate warming is of serious concern due to the unlikelihood of microevolution of nesting behavior keeping pace with climate change (Charmantier *et al.* 2008; McGaugh *et al.* 2009; Refsnider & Janzen 2015).

Understanding how geographically widespread species adjust to contemporary environmental variation can better enable researchers to predict how such species with environmentally-sensitive traits might accommodate rapidly changing environments. In the face of global climate change and other anthropogenic alterations of environments, it is critical to understand if nest-site choice is a viable strategy for organisms to keep pace with these changes. Future research should focus on examining the adaptive potential of the maternal behavior of nest-site choice. This adaptive potential could be investigated by determining the mechanisms of maintenance for nest-site choice across the species range and/or examining the relationships among nest-site choice, offspring phenotype, and fitness.

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## Table and Figures

Table 1: Comparisons of field locations including: latitude and longitude, mean historic July air temperature 1971-2000, substrate type, shade structures, and years of data collection.

<b>Name</b>	<b>State</b>	<b>Latitude Longitude</b>	<b>Mean July Air Temp</b>	<b>Substrate types</b>	<b>Shade structures</b>	<b>Years of data collection</b>
<b>Round Lake State Park</b>	Idaho	48.162586 °N -116.644995 °W	18.28	Loam and sand	Mostly mature coniferous trees with sparse deciduous trees	2013-2016
<b>Tamarac National Wildlife Refuge</b>	Minnesota	46.956219 °N -95.6483095 °W	19.95	Loam and gravel	Mature deciduous and coniferous trees; sparse grass prairie	2012; 2014-2015
<b>Smith and Bybee Wetland Natural Area</b>	Oregon	45.620349 °N -122.726986 °W	20.05	Loam and sand	Willow thickets and sparse deciduous trees	2014-2016
<b>Upper Mississippi River National Wildlife and Fish Refuge</b>	Illinois	41.94806 °N -90.116398 °W	22.67	Loam, gravel, and sand	Mature deciduous and coniferous trees	2013-2016
<b>Crescent Lake National Wildlife Refuge</b>	Nebraska	41.761369 °N -102.436622 °W	23.39	Loam, gravel, and sand	Various buildings and mature deciduous trees	2013-2015
<b>Bosque del Apache National Wildlife Refuge</b>	New Mexico	33.78189 °N -106.89174 °W	25.29	Sand and gravel	Willow thickets with various grasses and sedges	2014-2016

Table 2: Type III test of fixed effects of CTEs (°C) examining representative and natural nest sites across locations, years, and the interaction of location and year.

<b>Constant Temperature Equivalent Comparisons</b>		
Location	$F_{5,882}=118.89$	<b>p&lt;0.0001</b>
Nest Type	$F_{1,882}=340.60$	<b>p&lt;0.0001</b>
Year nested within Location	$F_{12,882}=6.97$	<b>p&lt;0.0001</b>
Location x Nest Type	$F_{5,882}=94.76$	<b>p&lt;0.0001</b>

Table 3: Type III test of fixed effects of CTEs (°C) examining natural nest sites across locations and years.

<b>Constant Temperature Equivalent Comparisons</b>		
Location	$F_{5,430}=44.85$	<b>p&lt;0.0001</b>
Year nested within Location	$F_{14,430}=13.90$	<b>p&lt;0.0001</b>

Table 4: Pairwise comparisons of least squares means of CTEs (°C) of natural nests, examining differences between specific years within a given location.

Location	Year	Year	Test Statistic
Idaho	2013	2014	p=0.1485
Idaho	2013	2015	p=0.6942
Idaho	2013	2016	<b>p&lt;0.0001</b>
Idaho	2014	2015	p=0.3112
Idaho	2015	2016	<b>p&lt;0.0001</b>
Minnesota	2012	2014	<b>p=0.0070</b>
Minnesota	2012	2015	<b>p=0.0452</b>
Minnesota	2014	2015	p=0.4741
Oregon	2014	2015	p=0.6281
Oregon	2014	2016	<b>p&lt;0.0001</b>
Oregon	2015	2016	<b>p&lt;0.0001</b>
Illinois	2013	2014	p=0.1587
Illinois	2013	2015	p=0.1261
Illinois	2013	2016	p=0.7108
Illinois	2014	2015	p=0.9196
Illinois	2014	2016	p=0.5772
Illinois	2015	2016	p=0.6090
Nebraska	2013	2014	<b>p&lt;0.0001</b>
Nebraska	2013	2015	<b>p&lt;0.0001</b>
Nebraska	2014	2015	p=0.1792
New Mexico	2014	2015	p=0.5871
New Mexico	2014	2016	p=0.0782
New Mexico	2015	2016	p=0.0649

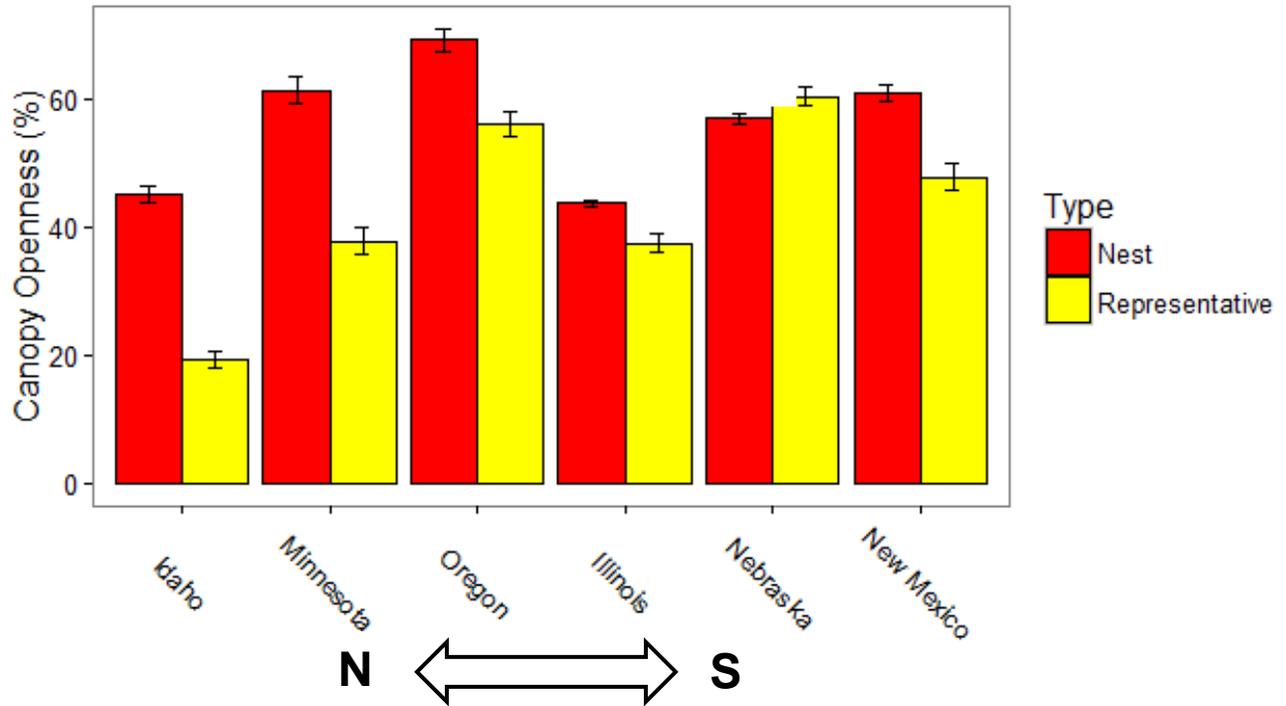


Figure 1: Least squares means comparisons of percent canopy openness between natural nests and representative sites across six locations.

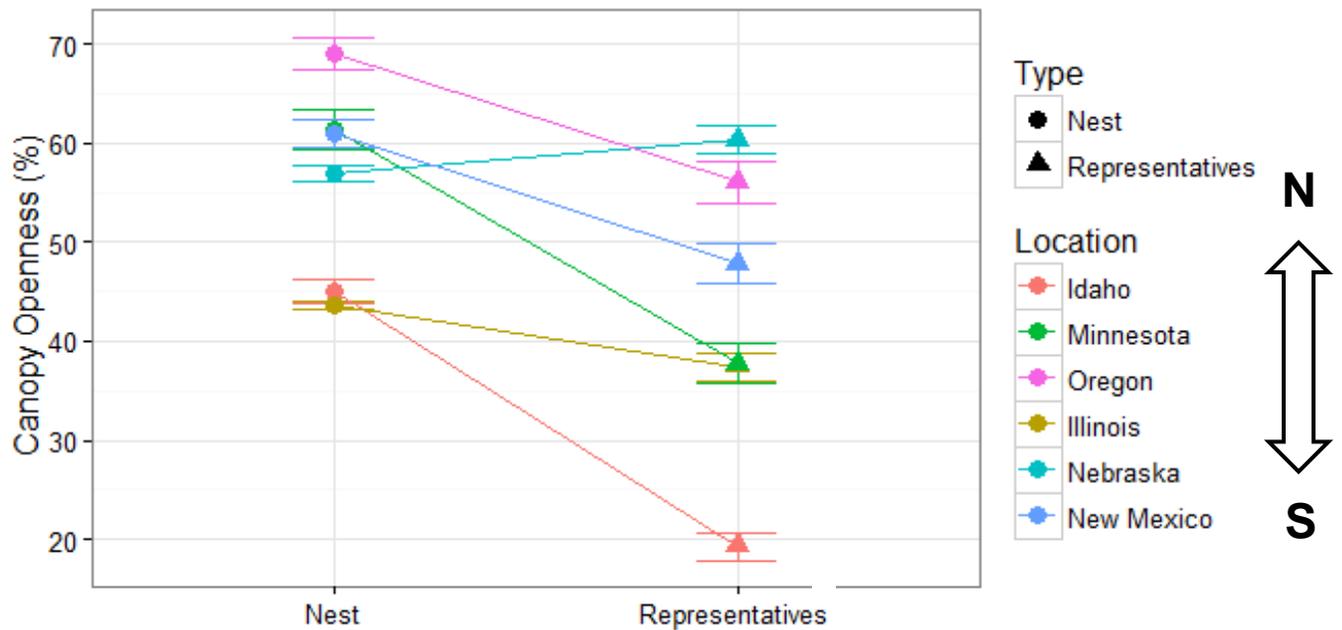


Figure 2: Least squares means comparisons of percent canopy openness between natural nests and representative sites across six locations, highlighting the interaction between location and nest type ( $F_{5,1810}=37.81, p<0.0001$ ).

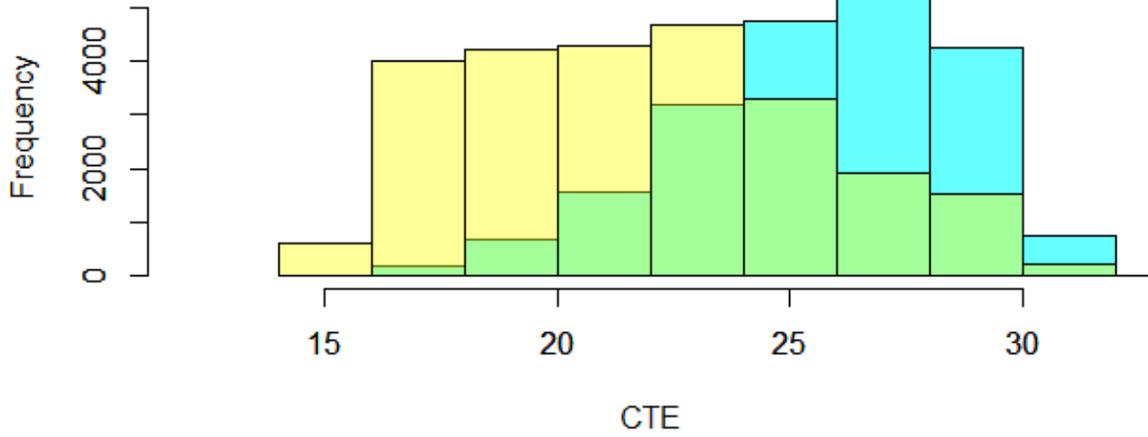


Figure 3: The distributions of the Constant Temperature Equivalents (CTEs) for the natural nests (blue) and the representative sites (yellow), and where natural nests and representative sites overlap (green).

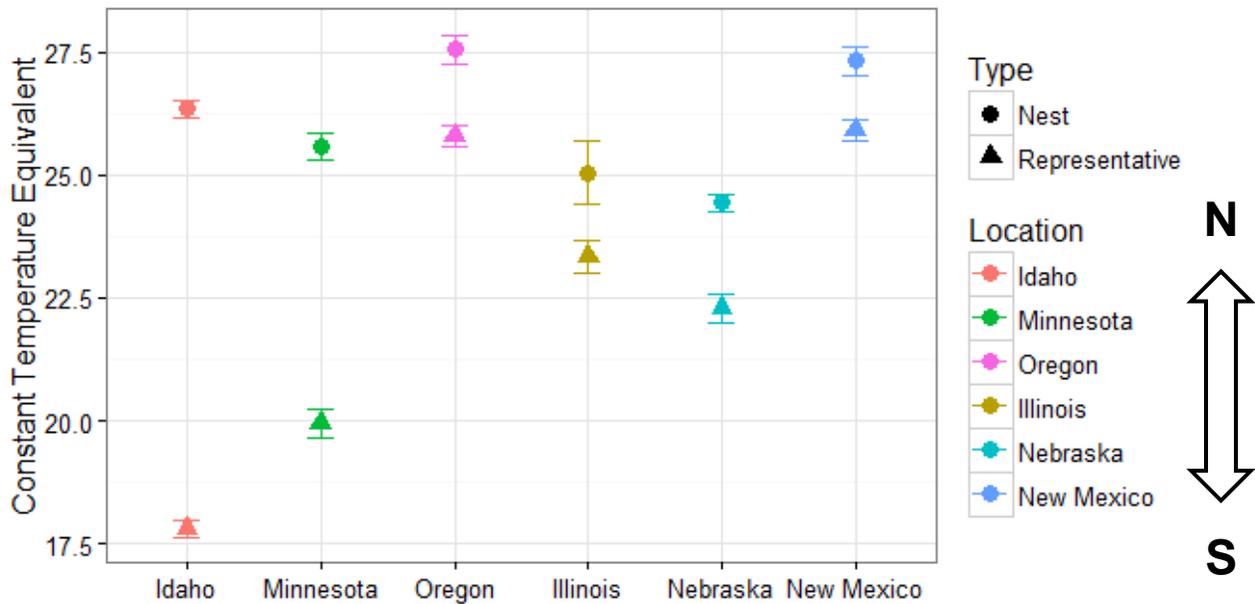


Figure 4: Least squares means comparisons of CTEs (in C°) between natural nests and representative sites across locations.

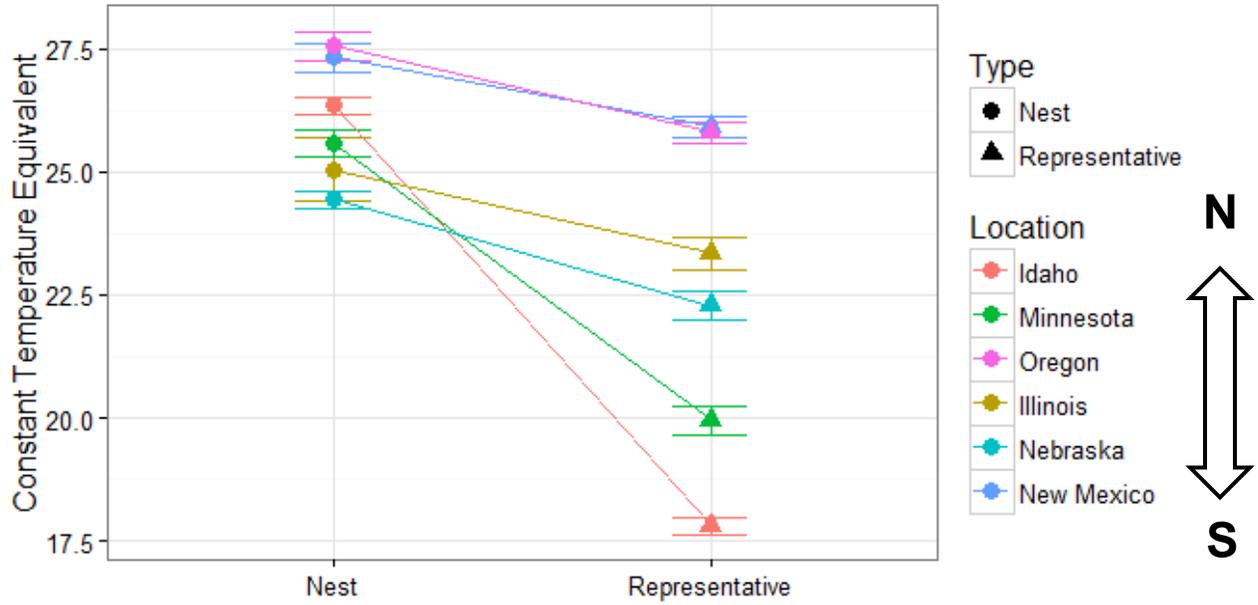


Figure 5: Least squares means comparisons of CTEs (in C°) between natural nests and representative sites across six locations, highlighting the interaction between location and type ( $F_{5,998}=88.41$ ,  $p<0.0001$ ).

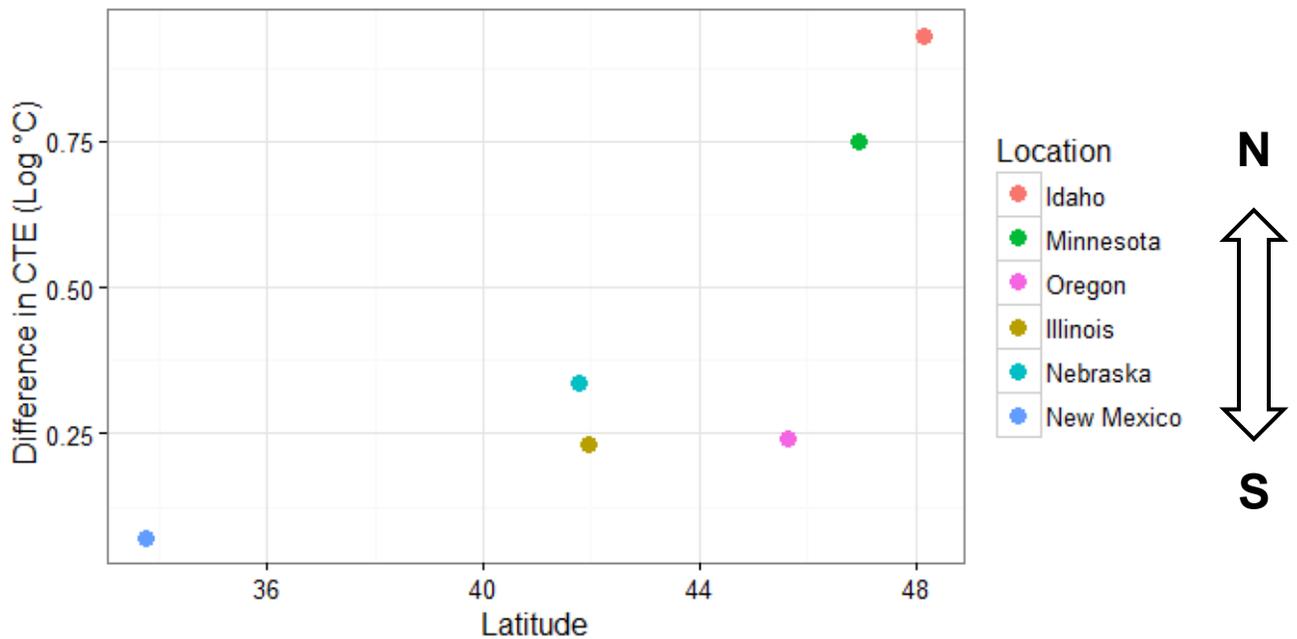


Figure 6: The log difference in mean CTEs between natural nests and representative sites of all locations across latitudes.

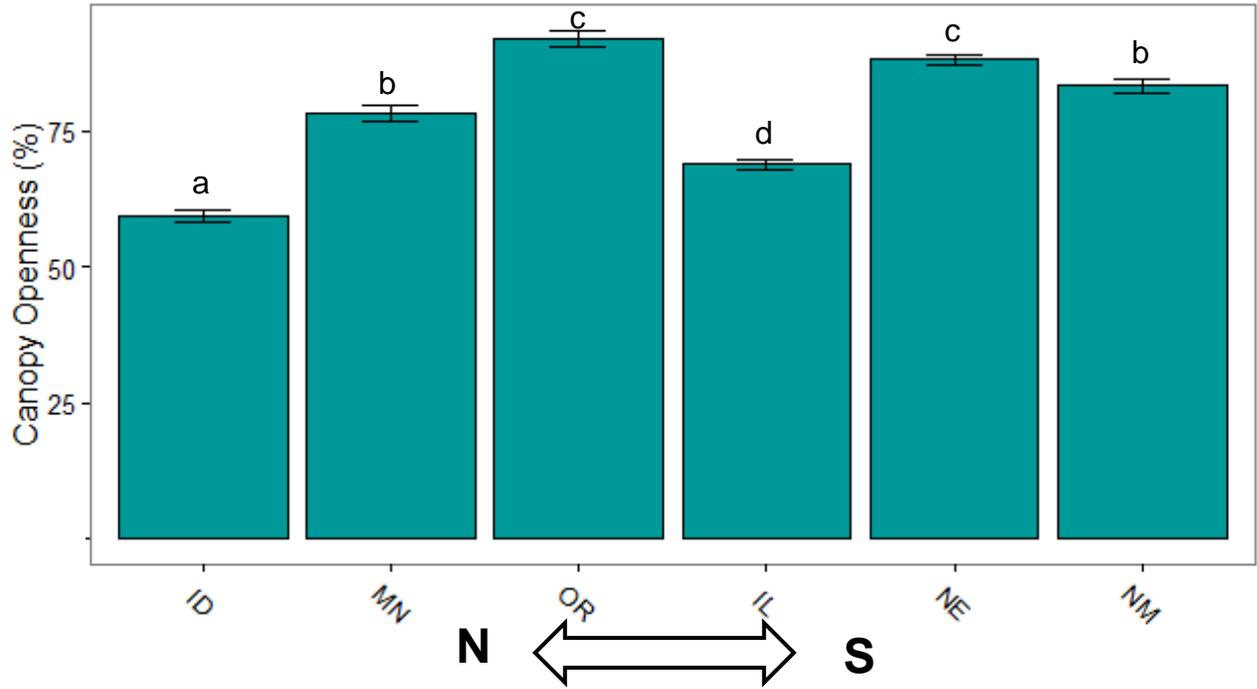


Figure 7: Least squares means comparisons of percent canopy openness between natural nests across six locations. Statistical difference among populations with different lower case letters at  $\alpha=0.05$ .

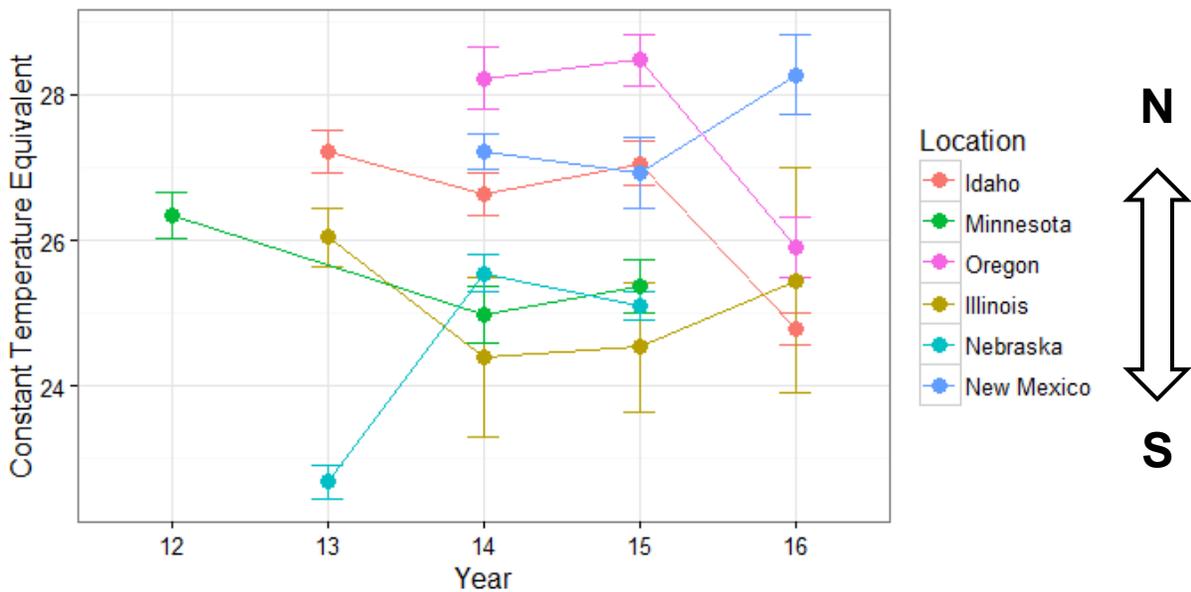


Figure 8: Least squares means comparisons of CTEs (in C°) of natural nests among years (2012-2016) across six locations.

## CHAPTER 3

GEOGRAPHIC VARIATION IN THERMAL SENSITIVITY OF EARLY LIFE TRAITS IN  
A WIDESPREAD REPTILE

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Keywords: Painted Turtle, *Chrysemys picta*, Geographic Variation, Local Adaptation

Abstract:

Organisms with large geographic distributions experience a great diversity of climatic conditions, requiring local adaptation and/or phenotypic plasticity. These eco-evolutionary processes are of particular interest in organisms with traits that are intrinsically connected to temperature, such as embryonic development in oviparous taxa. To examine potentially adaptive spatial patterns of phenotypic variation in a widespread vertebrate, we quantified fitness-related embryonic and hatchling traits of the painted turtle (*Chrysemys picta*) from seven locations across its geographic range (in Idaho, Minnesota, Oregon, Illinois, Nebraska, Kansas, and New Mexico). Eggs from these sites were incubated under constant conditions across a range of environmentally relevant temperatures. Thermal reaction norms for developmental rate, hatchling mass, carapace width, and survival to hatching varied among localities. We found

evidence to support local adaptation in some of these traits in common-garden conditions. However, latitude and the 30-year mean July air temperature (i.e., during the middle of development) for each location were not strong predictors of these geographic differences. These findings suggest that common proxies, such as latitude, used to encapsulate geographic patterns in phenotypic variation may not be reliable predictors. Thus, complex interactions between abiotic and biotic factors likely drive among-population phenotypic variation. Understanding spatial variation in key traits provides important perspective on current adaptation to climatic conditions and future phenotypic responses to projected climate change.

#### Introduction:

Understanding how species match phenotypes to their local environments by adaptation (Kawecki & Ebert 2004; Baumann & Conover 2011), phenotypic plasticity (Charmantier *et al.* 2008; Ballen, Shine & Olsson 2015), or some combination of the two (Crispo & Chapman 2010; Scheiner 2016), yields insights into these processes. In particular, species with broad geographic distributions are comprised of populations that experience different climates and, thus, are expected to match phenotypes to local environmental conditions across their ranges. Patterns of intraspecific variation among populations therefore can shed light on microevolutionary potential in response to environmental change.

Over the last century, scientists have devised a series of rules to describe geographic patterns of variation in biological diversity, body shape, body size, and other such phenomena (Mayr 1956; Stevens 1989; Rohde 1992). The basis for these rules ultimately derives from the expectation that processes associated with phenotypic variability, species ranges, and biodiversity are

thermally limited. Indeed, latitudinal gradients, frequently used as a proxy for temperature, have been good predictors for various phenotypic traits for many organisms across latitudinal clines (Iverson *et al.* 1993; Ashton 2002; Ashton & Feldman 2003). For example, in the Northern Hemisphere, lower latitudes are characterized by a longer growing season and earlier start dates due to a longer duration of warmer temperatures relative to higher latitudes (Conover & Present 1990; Worthen 1996; Frenne *et al.* 2013). Hence, latitude is frequently used as a predictor of macroclimatic conditions, and therefore as a predictor for phenotypic variation found in organisms ranging across latitudes (Ashton 2002; Stinchcombe *et al.* 2004). But to what extent are geographic patterns of macroclimatic conditions reflected in local genetic adaptation and/or phenotypic plasticity in the organisms found there? Answering this question is critical in the face of predicted changes in climate (IPCC 2014), specifically thermal alterations during the period of embryonic development that have the potential to affect organismal phenotypes and survival. Embryos of oviparous taxa are particularly sensitive because they lack post-parturition mechanisms for mitigating the environmental conditions to which they are exposed (Shine, Elphick & Harlow 1997; Refsnider & Janzen 2010; Telemeco *et al.* 2016). How will organisms with environmentally-sensitive traits persist under rapidly changing environmental conditions?

All biochemical reactions are thermally-dependent, but ectotherms are well known for having an inordinate fraction of their biology linked to prevailing thermal conditions. In turn, many of their vital rates and life-history traits that are influenced by temperature are inextricably associated with physiological processes (e.g., metabolic rates and digestive efficiency) (Huey 1982; Peterson, Gibson & Dorcas 1993; White, Phillips & Seymour 2006), behavior (Schieffelin & de Queiroz 1991; Keogh & DeSerto 1994; Mori & Burghardt 2001), and development (Bronikowski 2000; Birchard & Deeming 2004; Gangloff, Vleck & Bronikowski 2015),

including temperature-dependent sex determination in some species (Harrington Jr 1967; Bull 1983; Janzen & Paukstis 1988; Holleley *et al.* 2015). In many cases, free-living adult ectotherms alter habitat use to achieve optimal thermal conditions for these traits. Thus, oviparous organisms without parental care and with embryos that lack thermoregulatory ability during development (Telemeco *et al.* 2016) are especially vulnerable to local climatic conditions. Such vulnerabilities, and eco-evolutionary solutions to resolve or minimize them, can perhaps be most readily revealed among populations in taxa with broad geographic distributions.

These characteristics make the painted turtle, *Chrysemys picta* (Emydidae), an excellent system for investigating questions related to thermal sensitivities of early life stages. We compared phenotypes of developing *C. picta* from 7 locations (in Idaho, Minnesota, Oregon, Illinois, Nebraska, Kansas, and New Mexico) in a common-garden experiment. We tested the extent to which turtles across 14 degrees of latitude and concomitant climatic differences exhibit local adaptation and/or phenotypic plasticity for key early life traits. We employed a three-step statistical approach, whereby we first tested for the categorical effect of location, followed by the continuous effect of latitude, and then by mean July air temperature. We hypothesized that turtle embryos from higher latitude locations experience cooler nest temperatures in accord with well-known latitudinal clines in air temperatures. Therefore, we predicted that eggs from these areas would exhibit accelerated developmental rates, due to potential constraints of a shorter growing season, compared to eggs from more southern locations, when raised in a common-garden environment. Emydid turtles exhibit a pattern whereby adult females at higher latitudes produce larger clutch sizes of smaller eggs (Iverson *et al.* 1993). Therefore, we predicted that hatchlings from higher latitudes would have smaller body sizes than conspecifics from lower latitudes. The primary period of organogenesis for *C. picta* occurs during the middle of embryonic

development (Cordero & Janzen 2014), which roughly corresponds to July in the wild.

Therefore, we also predicted that painted turtles from locations that experience the warmest July air temperatures would exhibit fewer abnormalities and increased hatching success when incubated at higher incubation temperatures. Investigating the effects of developmental temperatures on phenotypes across multiple geographically widespread populations allows us to examine how these organisms are successful across greatly diverged contemporary climates. These insights could elucidate how these organisms will respond to a rapidly warming climate.

#### Methods:

*Study Organism:* The painted turtle (*Chrysemys picta*) occurs in many freshwater habitats from Mexico to Canada and the Pacific coast to the Atlantic coast (Starkey *et al.* 2003; Ernst & Lovich 2009). In late spring and early summer, females emerge from the water and lay shallow subterranean nests one to five times each reproductive season, with two clutches considered the norm. Each nest contains 1-23 eggs, with a mean of 12 eggs (Ernst & Lovich 2009). In field conditions, incubation typically lasts 55-85 days, with variation attributed primarily to temperature (Ernst & Lovich 2009; Refsnider 2016).

*Field and Lab Methods:* We used distinct populations from seven geographic locations across the species range for comparison, three of these locations being the focus of long-term research (Janzen, 1994; Iverson and Smith, 1993; Morjan, 2003; Table 1; Fig. 1). In late spring and early summer, we collected eggs from nests laid within 24 hours of oviposition at the field sites, 9-16 clutches per location, and transported them to Iowa State University for a common-garden incubation experiment. We weighed eggs, individually labeled them, and randomly assigned

them to incubation temperature treatments (26, 27, 27.5, 28, 28.5, 29, 29.5, and 30°C; Table 2). These temperatures span from the upper limit of viability in fine decrements to the middle of viable constant incubation temperatures (Ewert 1979; Ewert 1985). We split eggs from a clutch across treatments to account for potential maternal effects. Incubation temperature and substrate water potential (-150 kPa) remained constant throughout development, and with water being added weekly to the vermiculite to maintain moist hydric conditions, which are important for proper development (Gutzke & Packard 1985; Bodensteiner *et al.* 2015). Towards the end of incubation, we checked eggs daily for pipping (rupturing of the eggshell) and hatching. Once a hatchling fully emerged from its egg, we weighed the turtle, took linear measurements (carapace length, carapace width, and plastron length), and noted shell abnormalities, such as additional scutes (Telemeco *et al.* 2013).

*Statistical Methods:* We conducted all statistical analyses in SAS (SAS Institute, Cary, NC) using generalized linear mixed models with the MIXED procedure to compare turtle phenotypes among locations and incubation temperatures. We tested effects and interactions of biological interest, keeping the models identical across dependent variables to allow for direct comparisons of effect sizes. We examined response variables (incubation duration, hatchling mass, carapace length, carapace width, and plastron length) in a reduced model framework. The final model contained Location + Temperature + Location  $\times$  Incubation Temperature + Incubation Temperature<sup>2</sup>, initial egg mass as a covariate, and the random effect of clutch. We included the quadratic term, Incubation Temperature<sup>2</sup>, in our models because temperature and development are related non-linearly (Sokal & Rohlf 1981; Janzen 1993). Additionally, because of unbalanced groups, we used the Satterthwaite degrees of freedom correction (Littell *et al.* 2006).

We performed statistical analyses in a three-step process. Initially, we employed a mixed-effect model using restricted maximum likelihood with clutch nested within location as a random effect to address the influence of temperature and location on the phenotypes of interest. Overall, we treated lab incubation temperature (T), temperature<sup>2</sup> (T<sup>2</sup>), and initial egg mass as continuous fixed effects and location as a categorical fixed effect. For the second and third steps in our analytical process, we re-ran these models with latitude as a continuous fixed effect in place of location and then again with the 30-year mean July air temperature (T<sub>mat</sub>) (i.e., during the middle of organogenesis) as a continuous fixed effect in place of location. We acquired historic climate data from the National Climate Data Center weather station nearest each field location ([www.ncdc.noaa.gov](http://www.ncdc.noaa.gov)). To further assess potential among-location variation in thermal sensitivity, we used the model above while restricting the data set to 27.5°C and 28.5°C (where all locations were represented; Table 2).

In addition to the continuous dependent variables described above, we tested for differences in hatching success and frequency of abnormalities across incubation temperatures. In both cases, we used a binomial logistic regression with a logit-link function:  $Y \sim \text{Location} + \text{Incubation Temperature} + \text{Location} \times \text{Incubation Temperature}$  and the random effect of clutch, with the Satterthwaite degrees of freedom correction (Littell *et al.* 2006). Kansas was removed from the analysis of hatching success due to high egg mortality, potentially because females were induced with oxytocin to lay these eggs before they were ready, a method not used for any other location. We only used this method in Kansas because we were unable to locate nests at this location.

## Results

All traits measured varied under common-garden conditions in the laboratory as a function of the location of origin and the thermal incubation environment experienced. Most eggs hatched (588 out of 891; excluding Kansas, 541 out of 647) and few offspring exhibited obvious abnormalities (103 out of 588). Incubation duration declined as a function of incubation temperature and ranged from 43-69 days, with a mean of 57 days. Initial egg mass positively and strongly predicted all four measures of body size ( $R^2$  values ranging from 0.40-0.72). Regardless, measures of body size varied considerably. Hatchling mass ranged from 2.90-6.94 g, with a mean of 4.85 g. The carapace length of hatchlings ranged from 20.89-36.40 mm, with a mean of 25.84 mm. Plastron length and carapace width were less variable measures, ranging from 19.18-28.81 mm and 17.10-26.00 mm, respectively.

### Developmental Rate

Incubation duration differed between locations ( $F_{6, 540}=6.22$ ,  $p<0.0001$ ). As anticipated, incubation duration declined with increasing constant incubation temperatures  $T$  and  $T^2$ , and embryos from different locations responded differently to  $T$  (Table 3), with embryos from New Mexico and Nebraska exhibiting especially fast development rates at any given temperature (Fig. 2). Despite these substantive effects of location on incubation duration, neither latitude nor 30-year  $T_{\text{mat}}$  were good predictors when substituted into the model for location (Table 3).

### Hatchling Body Size

As expected, initial egg mass was the primary driver of size at hatching, positively predicting most of the variation in all four measures of body size. As with incubation duration, we detected differences in egg mass-adjusted body size between locations (Figs. 3-6), although this

geographic variation only yielded statistically significant results for hatchling mass and carapace width (Tables 4-7). Of particular note, hatchling mass from Oregon tended to decline with increasing T in contrast to the other locations (Fig. 3). Hatchling carapace width tended to decrease with increasing T, apart from Kansas hatchlings, which reversed that pattern (Fig. 5). Carapace length and plastron length did not differ among locations (Fig. 4 and 6). Once again, latitude and  $T_{\text{mat}}$  were not good predictors of body size (Tables 4-7), with one exception (Table 4): latitude was more positively related to hatchling mass at lower T than at higher ones (Fig. 3). To identify whether phenotypic differences among locations were best explained by intrinsic (e.g., genetic) factors or phenotypic plasticity, we tested for differences among locations within two T (common-garden conditions at 27.5°C and 28.5°C where all locations were represented). We detected differences among all seven locations within each incubation regime, indicating heritable genetic factors and/or pre-ovulatory maternal effects accounting for differences in phenotypes (Table 8 and 9). Notably, compared to those from other locations, embryos from Nebraska developed particularly fast and were heavier at hatching after accounting for egg mass, whereas offspring from Minnesota were longer and wider on average.

#### *Abnormalities and Hatching Success*

For hatchling abnormalities, we detected no effect of T, location, or their interaction (all  $P > 0.10$ ; Table 10). Even though the main effect of location was not statistically significant, Oregon offspring had a reduced probability of having an abnormality (Fig. 7). In contrast, location and its interaction with T affected hatching success, seemingly driven by the increased mortality of New Mexico and Nebraska embryos (Fig. 8). Our prediction of southern populations being more resilient to increased T was not supported; markedly, embryos from Nebraska had a reduced

probability of surviving to hatching as T increased, whereas New Mexico embryos displayed the opposite pattern (Fig. 9).

## Discussion

Geographically widespread species are likely to experience divergent environmental conditions across their range and frequently exhibit phenotypic responses to match those environmental differences via some combination of local adaptation or phenotypic plasticity (Kawecki & Ebert 2004; Charmantier *et al.* 2008; Baumann & Conover 2011; Ballen, Shine & Olsson 2015; Scheiner 2016). Elucidating the responses to current thermal variation of geographically widespread organisms with thermally-sensitive traits could give researchers a greater understanding of microevolutionary responses to environmental change. We found variation in thermal reaction norms among our widely-distributed study locations of painted turtles, but this variation could not be attributed to latitude or to different measures of local climatic conditions. Even so, we detected evidence under common-garden conditions in the laboratory consistent with local adaptation for nearly all traits of neonatal turtles we measured, including incubation duration, hatchling mass, and linear body measurements.

Our prediction that eggs from higher latitude locations would develop faster when compared with eggs from more southern locations when incubated in common-garden conditions was not supported. We detected differences between our locations, but neither latitude nor the typical mean July air temperature were good predictors of patterns found in this study. Incubation duration is of particular interest because painted turtle hatchlings overwinter in the nest across their range. Thus, for example, if offspring were to utilize more of their yolk to develop quickly

and remain in the nest during the warmer temperatures of late summer and fall, this could be problematic for fitness during the overwintering and dispersal life stages. Such hatchlings would presumably utilize more of their residual yolk and body stores due to increased metabolic expenditure at higher temperatures (Huey 1982; Willette, Tucker & Janzen 2005; Muir *et al.* 2013). With predicted increases in temperatures, especially during winter at northern latitudes, current developmental life-history strategies could become detrimental, disproportionately impacting our New Mexico and Nebraska populations.

According to Bergmann's Rule, as environmental temperatures decrease, often along latitudinal or elevational gradients, then animal body size increases (Mayr 1956). Support for this maxim is found in mammals, birds, and some other reptilian lineages (Ashton, Tracy & Queiroz 2000; Ashton 2002; Ashton, Feldman & Garland Jr 2003). Evidence supports that turtles follow Bergmann's Rule (Ashton and Feldman 2003), such that mean asymptotic body size is larger at higher latitudes and these larger body sizes are associated with variation in life-history traits (e.g., increased time to sexual maturity and larger clutch sizes) (Iverson *et al.* 1993). In this study, our hypothesis was not supported: the measures of hatchling body size – mass, carapace length, carapace width, and plastron length – were not smaller in our more northern populations and larger in more southern populations. Adjusting for initial egg mass, we detected no clinal variation of any kind in our measures of offspring body size, besides the effect of latitude and the interaction between T and latitude on hatchling mass that seems to be driven by lower incubation temperatures (Fig. 3). However, we did find evidence that hatchling mass and carapace width differed between locations, lending support to the potential of these differences being attributable to microenvironmental conditions rather than macroenvironmental patterns.

We predicted that eggs from the more southern populations that experience the warmest air temperatures during key stages of development would be more resilient to increased T, therefore having fewer morphological abnormalities and increased hatching success relative to presumably cooler northern populations. Extreme T can increase probability of offspring morphological abnormalities (Telemeco et al. 2013), and these abnormalities may be negatively correlated to fitness. In long term study populations, morphological abnormalities are present in much higher levels in young than in adults, suggesting that selection eliminates many offspring with abnormalities before they reach adulthood (Arnold & Peterson 2002; Telemeco *et al.* 2013). Also, T and hatching success are often inversely correlated, such that eggs incubated at the highest T are more likely to fail to hatch (Gutzke & Packard 1985; Packard, Packard & Birchard 1989; Van Damme *et al.* 1992). However, we found no effect of T, location, or their interaction on presence of abnormalities. In contrast, hatching success varied among locations as a function of T and location. New Mexico embryos had a decreased probability of successfully hatching at lower T, whereas Nebraska embryos had a decreased probability of successfully hatching at higher T; embryos from the other locations had a high probability of hatching successfully across all T. The reduced resilience of New Mexico embryos at cooler T may be consistent with our predictions, but the findings for Nebraska embryos are puzzling without corresponding results for embryos from the considerably more northern study locations in Idaho, Minnesota, and Oregon.

Due to the overall lack of clinal variation detected in the study, we sought alternative environmental variables that might explain the observed variation in phenotypes among locations. Therefore, we explored the 30-year variance in mean July air temperature and longitude, as neither the 30-year mean July air temperature nor latitude were good predictors of

differences for most phenotypes. Another factor that could be contributing to variation in phenotypes that was not investigated in this study is varying T during incubation. For example, variance in T affects development rate of reptilian embryos under lab and field conditions (Shine & Harlow 1996; Ashmore & Janzen 2003). As the variance in T increases, so does the likelihood that some portion of the daily T dips below the lower threshold for embryonic development or exceeds the critical thermal maximum, and therefore could affect rates of development and resulting phenotypic variation. To illustrate, an increase in the thermal variance in the lab can increase the developmental period (Ashmore & Janzen 2003), yet the sensitivity to thermal variance can vary among populations or species (e.g.-(Andrews, Mathies & Warner 2000). Rate of development and T thus have a complex relationship, so variance in T could be important to consider in explaining among-population patterns of phenotypic variation.

#### Local adaptation and phenotypic plasticity

It is generally understood that populations of organisms with broad geographic distributions should adjust to local biotic and abiotic conditions via adaptation, phenotypic plasticity, or some combination of the two. In general, the expectation is that organisms with faster generation times are more likely to achieve a fit to local conditions through adaptation rather than via phenotypic plasticity. Abundant evidence exists to support this contention for short-lived organisms (*Drosophila* (Huey *et al.* 2000; Gilchrist, Huey & Serra 2002), pitcher-plant mosquitoes (Bradshaw & Holzapfel 2008), silversides (Conover & Heins 1987), *Arabidopsis* (Fournier-Level *et al.* 2011), and bacteria (Vos *et al.* 2009)). Long-lived organisms such as turtles might instead be more prone to adjust to local conditions, at least initially, through phenotypic

plasticity because of their long generation times relative to the pace at which local conditions change (*sensu* (Refsnider & Janzen 2016)).

We found that thermal reaction norms for all traits varied among our widely-distributed study locations of painted turtles. This variation could not be explained by latitude or different measures of local climatic conditions, unlike in many systems where latitudinally-linked climatic conditions explain the patterns of certain traits observed in nature (David & Bocquet 1975; Coyne & Beecham 1987; Bradshaw & Holzapfel 2008). Moving forward with work of this nature, interpretations should be considered with caution due to the complexity of natural systems. Other aspects of microclimate variation that may drive differences among locations in our system include precipitation/hydric conditions (Bodensteiner *et al.* 2015), nest-shade cover (Janzen 1994), gaseous environment (Cordero *et al.* 2017), and/or regional weather patterns.

Regardless of the source(s) of selection, we detected evidence consistent with local adaptation for nearly all traits of neonatal turtles we measured under common-garden conditions. That is, phenotypic differences among all seven locations often persisted even when embryos were reared under identical thermal and hydric conditions. Still, a tension between the explanations of local adaptation and phenotypic plasticity is evident for other traits in turtles and suggests their coexistence and even synergy. For example, (Ewert, Lang & Nelson 2005) and (Schroeder *et al.* 2016) provide evidence of local adaptation for thermal sensitivity of temperature-dependent sex determination (TSD) among conspecific turtle populations arrayed from north to south in the United States, whereas (Refsnider & Janzen 2012) detected considerable phenotypic plasticity in a common-garden field experiment for TSD-related nesting behavior among five populations of painted turtles, including three used as sources in our study. Moreover, in examining among-clutch responses to T, we found evidence consistent with genetic by environmental (GxE) effects

for certain phenotypes. These GxE effects seem to be primarily driven by the Nebraska population, warranting more careful study in the future.

One explanation for the modest geographic patterns of phenotypic differentiation found here is that nest-site choice could buffer ambient climatic conditions via selection of specific microhabitats within each location (Bernardo 1996; Refsnider & Janzen 2010). Such nest-site choice can be major factor affecting offspring phenotype and survival in a variety of taxa (Resetarits 1996; Brown & Shine 2004; Ewert, Lang & Nelson 2005; Refsnider 2016), especially in those that lack parental care, where developing embryos are exposed to the abiotic and biotic conditions of the environment in which they are oviposited (Mitchell, Warner & Janzen 2013; Mitchell, Maciel & Janzen 2015). Nest-site choice in *C. picta* is a heritable behavior under certain environmental conditions, and therefore microevolution of this trait could occur (McGaugh *et al.* 2009). However, its potential to maximize maternal and offspring fitness might be driven more by plasticity of this trait (Kamel & Mrosovsky 2006; Refsnider & Janzen 2012; Refsnider & Janzen 2016). Plasticity in nest-site choice could be mitigating macroclimatic differences among these locations, and therefore could explain why latitude and typical mean July air temperature were not good predictors of the differences we observed in among-location thermal reaction norms for phenotypic variation of the offspring.

It is imperative to understand how species are accommodating the environment and how they are being affected by a rapidly changing climate. To do so, we have been characterizing the spatial and temporal microhabitats of many populations of painted turtles in the field. Behavioral plasticity may be mitigating the impacts of changing climatic conditions, yet we identified evidence of adaptation of embryos from different locations to the thermal environment in which they develop. These populations have had thousands of years to locally adapt to differing

climates post-Pleistocene glaciation (Starkey *et al.* 2003). Thus, a major concern is the rate at which the global climate is warming; many long-lived organisms may not be able to keep pace with the rapidity of these environmental changes (Visser 2008; Refsnider & Janzen 2016). This challenge is especially pertinent in species that have key life-history traits intrinsically tied to temperature, and are at the mercy of environmental conditions during development, lacking the ability to behaviorally or otherwise compensate for unfavorable circumstances.

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## Tables and Figures

Table 1: Name, coordinates, elevation (m), historic (1971-2000) mean July air temperature (C°), sample size, and year in which eggs were collected from each field location.

Location	Latitude and Longitude	Elevation	Mean July Air Temperature	Hatchlings Measured	Years of Egg Collection
Round Lake State Park, ID	48.162586° N, - 116.644995° W	661	18.28	n=129	2013
Tamarac National Wildlife Refuge, MN	46.956219° N - 95.6483095° W	452	19.95	n=66	2012
Smith and Bybee Wetlands Natural Area, OR	45.620349° N, - 122.726986° W	159	20.05	n=74	2014
Thomson Causeway Recreation Area, IL	41.94806° N, - 90.116398° W	192	22.67	n=137	1998
Crescent Lake National Wildlife Refuge, NE	41.761369° N, - 102.436622° W	1160	23.39	n=123	2010
Ross Natural History Preserve, KS	38.497202° N, - 96.336508° W	383	25.91	n=28	2013
Bosque del Apache National Wildlife Refuge, NM	33.78189° N, - 106.89174° W	1384	25.29	n=31	1998

Table 2: Schematic of experimental design, with “X” denoting successful incubation and hatching at this incubation temperature and location combination. Empty cells indicate temperature x location combinations that were not explored.

Incubation Temperatures  Locations	26°C	27°C	27.5°C	28°C	28.5°C	29°C	29.5°C	30°C
Idaho	X	X	X	X	X	X		X
Minnesota	X	X	X	X	X	X		X
Oregon	X	X	X	X	X	X		X
Illinois			X	X	X	X	X	
Nebraska	X	X	X	X	X	X		X
Kansas	X	X	X	X	X	X		
New Mexico			X		X		X	

Table 3: Three-step process for mixed models evaluating incubation duration in days (see text for details).

	<b><u>Incubation Duration (days)</u></b>		
	<b><u>Location Descriptor</u></b>		
	<b>Location (Categorical)</b>	<b>Location Latitude</b>	<b>Location Mean July Air Temp</b>
<i>Location</i>	F <sub>6, 540</sub> = 6.22 <b>p&lt;0.0001</b>	F <sub>1, 550</sub> =0.27 p=0.6012	F <sub>1, 554</sub> =0.00 p=0.9964
<i>Temperature</i>	F <sub>1, 521</sub> =83.20 <b>p&lt;0.0001</b>	F <sub>1, 526</sub> =82.79 <b>p&lt;0.0001</b>	F <sub>1, 525</sub> =85.79 <b>p&lt;0.0001</b>
<i>Temperature</i> <sup>2</sup>	F <sub>1, 521</sub> =68.23 <b>p&lt;0.0001</b>	F <sub>1, 526</sub> =74.25 <b>p&lt;0.0001</b>	F <sub>1, 525</sub> =73.56 <b>p&lt;0.0001</b>
<i>Temperature x Location</i>	F <sub>6, 515</sub> =6.58 <b>p&lt;0.0001</b>	F <sub>1, 523</sub> =0.72 p=0.3971	F <sub>1, 522</sub> =0.10 p=0.7568
<i>Initial Egg Mass</i>	F <sub>1, 567</sub> =2.10 p=0.1478	F <sub>1, 564</sub> =74.25 <b>p=0.0049</b>	F <sub>1, 566</sub> =7.55 <b>p=0.0062</b>

Table 4: Three-step process for mixed models evaluating hatchling mass in grams (see text for details).

	<b>Hatchling Mass (g)</b>		
	<b>Location Descriptor</b>		
	<b>Location (Categorical)</b>	<b>Location Latitude</b>	<b>Location Mean July Air Temp</b>
<i>Location</i>	F <sub>6, 536</sub> = 4.96 <b>p&lt;0.0001</b>	F <sub>1, 549</sub> =5.40 <b>p=0.0205</b>	F <sub>1, 550</sub> =2.01 p=0.1568
<i>Temperature</i>	F <sub>1, 532</sub> =1.89 p=0.1701	F <sub>1, 541</sub> =2.74 p=0.0982	F <sub>1, 538</sub> =0.55 p=0.4585
<i>Temperature</i> <sup>2</sup>	F <sub>1, 532</sub> =2.05 p=0.1525	F <sub>1, 539</sub> =1.50 p=0.2213	F <sub>1, 539</sub> =1.18 p=0.2770
<i>Temperature x Location</i>	F <sub>6, 522</sub> =5.08 <b>p&lt;0.0001</b>	F <sub>1, 536</sub> =5.10 <b>p=0.0244</b>	F <sub>1, 534</sub> =1.78 p=0.1826
<i>Initial Egg Mass</i>	F <sub>1, 568</sub> =769.98 <b>p&lt;0.0001</b>	F <sub>1, 475</sub> =854.75 <b>p&lt;0.0001</b>	F <sub>1, 480</sub> =844.92 <b>p&lt;0.0001</b>

Table 5: Three-step process for mixed models evaluating carapace length of hatchlings in millimeters (see text for details).

	<u>Carapace Length (mm)</u>		
	<u>Location Descriptor</u>		
	<b>Location (Categorical)</b>	<b>Location Latitude</b>	<b>Location Mean July Air Temp</b>
<i>Location</i>	F <sub>6,535</sub> = 0.95 p=0.4583	F <sub>1,549</sub> =0.03 p=0.8651	F <sub>1, 551</sub> =0.55 p=0.4587
<i>Temperature</i>	F <sub>1, 543</sub> =3.18 p=0.0753	F <sub>1, 548</sub> =3.32 p=0.0692	F <sub>1, 552</sub> =5.01 <b>p=0.0256</b>
<i>Temperature</i> <sup>2</sup>	F <sub>1, 543</sub> =3.37 p=0.0671	F <sub>1, 547</sub> =4.18 <b>p=0.0414</b>	F <sub>1, 553</sub> =4.71 <b>p=0.0304</b>
<i>Temperature x Location</i>	F <sub>6, 528</sub> =1.04 p=0.3997	F <sub>1, 542</sub> =0.03 p=0.8655	F <sub>1, 546</sub> =0.59 p=0.4445
<i>Initial Egg Mass</i>	F <sub>1,494</sub> =323.92 <b>p&lt;0.0001</b>	F <sub>1, 547</sub> =370.96 <b>p&lt;0.0001</b>	F <sub>1,553</sub> =292.26 <b>p&lt;0.0001</b>

Table 6: Three-step process for mixed models evaluating plastron length of hatchlings in millimeters (see text for details).

	<b>Plastron Length (mm)</b>		
	<b>Location Descriptor</b>		
	<b>Location (Categorical)</b>	<b>Location Latitude</b>	<b>Location Mean July Air Temp</b>
<i>Location</i>	F <sub>6,535</sub> = 1.54 p=0.1620	F <sub>1, 550</sub> =0.01 p=0.9410	F <sub>1, 549</sub> =0.13 p=0.7224
<i>Temperature</i>	F <sub>1, 542</sub> =4.03 <b>p=0.0453</b>	F <sub>1, 552</sub> =5.40 <b>p=0.0205</b>	F <sub>1, 548</sub> =6.27 <b>p=0.0126</b>
<i>Temperature</i> <sup>2</sup>	F <sub>1, 542</sub> =4.21 <b>p=0.0406</b>	F <sub>1, 550</sub> =6.47 <b>p=0.0113</b>	F <sub>1, 549</sub> =6.36 <b>p=0.0120</b>
<i>Temperature x Location</i>	F <sub>6, 528</sub> =1.39 p=0.2154	F <sub>1, 544</sub> =0.00 p=0.9962	F <sub>1, 542</sub> =0.18 p=0.6750
<i>Initial Egg Mass</i>	F <sub>1,491</sub> =362.99 <b>p&lt;0.0001</b>	F <sub>1, 328</sub> =418.59 <b>p&lt;0.0001</b>	F <sub>1,338</sub> =413.30 <b>p&lt;0.0001</b>

Table 7: Three-step process for mixed models evaluating carapace width of hatchlings in millimeters (see text for details).

	<u>Carapace Width (mm)</u>		
	<u>Location Descriptor</u>		
	<b>Location (Categorical)</b>	<b>Location Latitude</b>	<b>Location Mean July Air Temp</b>
<i>Location</i>	F <sub>6,534</sub> = 2.16 <b>p=0.0455</b>	F <sub>1, 548</sub> =0.00 p=0.9589	F <sub>1, 549</sub> =0.15 p=0.7010
<i>Temperature</i>	F <sub>1, 536</sub> =0.02 p=0.8911	F <sub>1, 539</sub> =0.50 p=0.4787	F <sub>1, 564</sub> =1.22 p=0.2695
<i>Temperature</i> <sup>2</sup>	F <sub>1, 536</sub> =0.00 p=0.9748	F <sub>1, 538</sub> =0.85 p=0.3563	F <sub>1, 566</sub> =1.53 p=0.2167
<i>Temperature x Location</i>	F <sub>6, 524</sub> =2.37 <b>p=0.0286</b>	F <sub>1, 534</sub> =0.01 p=0.9242	F <sub>1, 557</sub> =0.21 p=0.6437
<i>Initial Egg Mass</i>	F <sub>1,539</sub> =185.04 <b>p&lt;0.0001</b>	F <sub>1, 480</sub> =190.06 <b>p&lt;0.0001</b>	F <sub>1,192</sub> =65.70 <b>p&lt;0.0001</b>

Table 8: Painted turtle responses for eggs incubated at a constant temperature of 27.5°C.

	Idaho (n=18)	Minnesota (n=19)	Oregon (n=11)	Illinois (n=27)	Nebraska (n=21)	Kansas (n=9)	New Mexico (n=10)
Incubation Duration (days)*	60.94± 0.85	58.74± 0.84	59.21± 1.03	59.72± 0.76	55.23± 0.78	60.14± 1.12	55.98± 1.31
Hatchling Mass (g)*	4.91± 0.12	4.78± 0.12	4.73± 0.15	4.97± 0.10	5.11± 0.12	4.30± 0.16	4.47± 0.18
Carapace Length (mm)*	25.45± 0.28	26.55± 0.27	25.90± 0.33	26.29± 0.23	25.47± 0.29	24.71± 0.38	25.90± 0.40
Plastron Length (mm)*	24.79± 0.30	25.44± 0.29	23.73± 0.37	25.14± 0.24	24.68± 0.32	24.07± 0.42	24.62± 0.41
Carapace Width (mm)*	22.64± 0.30	24.36± 0.28	23.32± 0.36	24.57± 0.24	22.72± 0.31	22.53± 0.41	22.99± 0.41

Values shown are LSM±SE

\*Significant difference among populations at  $\alpha=0.05$

Table 9: Painted turtle responses for eggs incubated at a constant temperature of 28.5°C. given location and incubation temperature, with initial egg mass as a covariate.

	Idaho (n=19)	Minnesota (n=19)	Oregon (n=11)	Illinois (n=29)	Nebraska (n=21)	Kansas (n=9)	New Mexico (n=10)
Incubation Duration (days)*	56.93± 0.71	53.02± 0.92	55.43± 0.86	56.36± 0.63	53.31± 0.66	56.50± 1.34	53.18± 1.08
Hatchling Mass (g)*	4.88± 0.13	4.41± 0.16	4.54± 0.15	4.91± 0.12	4.97± 0.13	4.71± 0.24	4.47± 0.19
Carapace Length (mm)	25.56± 0.28	26.24± 0.34	25.20± 0.33	26.00± 0.23	25.13± 0.29	26.02± 0.53	25.87± 0.39
Plastron Length (mm)*	24.53± 0.27	25.00± 0.33	23.60± 0.31	24.84± 0.22	24.39± 0.27	24.88± 0.50	24.20± 0.38
Carapace Width (mm)*	22.67± 0.33	23.62± 0.40	23.57± 0.38	24.14± 0.27	22.57± 0.33	23.62± 0.61	22.69± 0.47

Values shown are LSM±SE

\*Significant difference among populations at  $\alpha=0.05$

Table 10: Effects of location and incubation temperature ( $^{\circ}\text{C}$ ) on the probability of a hatchling painted turtle having an abnormality.

Probability of Abnormalities Present in Hatchlings	
<i>Temperature</i>	$F_{1,574}=0.57,$ $p=0.4507$
<i>Location</i>	$F_{6,574}=1.58,$ $p=0.1518$
<i>Temperature x Location</i>	$F_{6,574}=1.64,$ $p=0.1350$

Table 11: Effects of location and incubation temperature ( $^{\circ}\text{C}$ ) on the probability of a painted turtle embryo surviving to hatch.

Probability of Hatching Success	
<i>Temperature</i>	$F_{1,548}=0.27,$ $p=0.6004$
<i>Location</i>	$F_{5,548}=2.13,$ $p=0.0610$
<i>Temperature x Location</i>	$F_{5,548}=2.51,$ $p=0.0294$

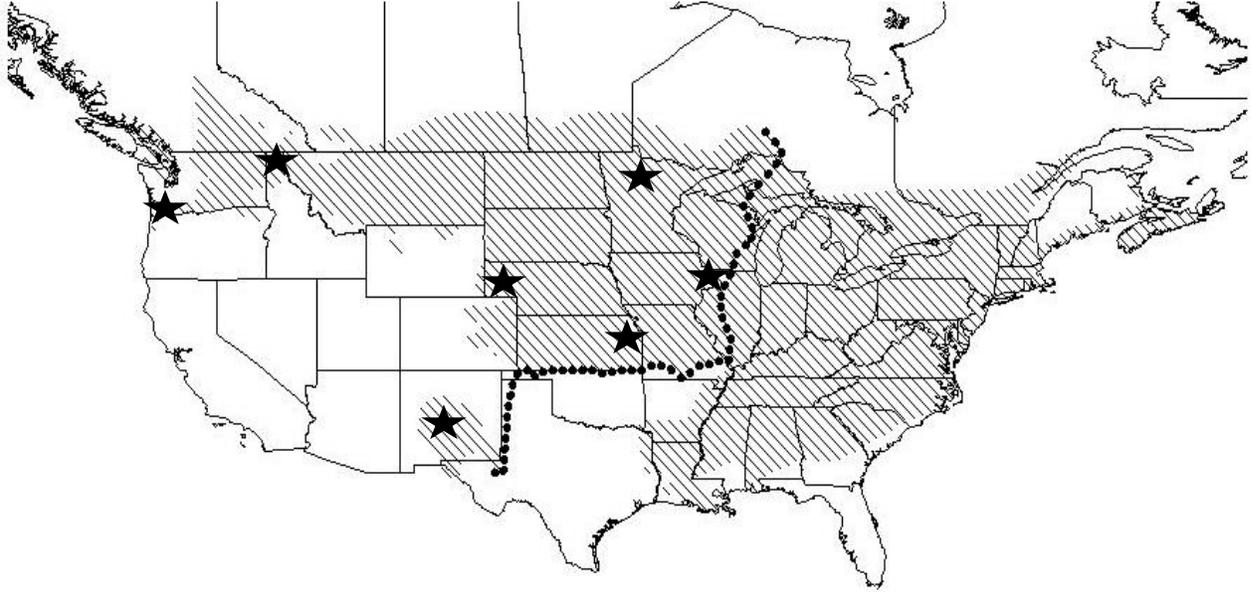


Figure 1: Modification of the figure from Refsnider et al. 2014, where the shaded region is the geographic range of *C. picta*. Stars denote field locations across the western United States (located in Idaho, Minnesota, Oregon, Illinois, Nebraska, Kansas, and New Mexico). To the left of the dashed line represents the range of the western sub-species, *Chrysemys picta belli*.

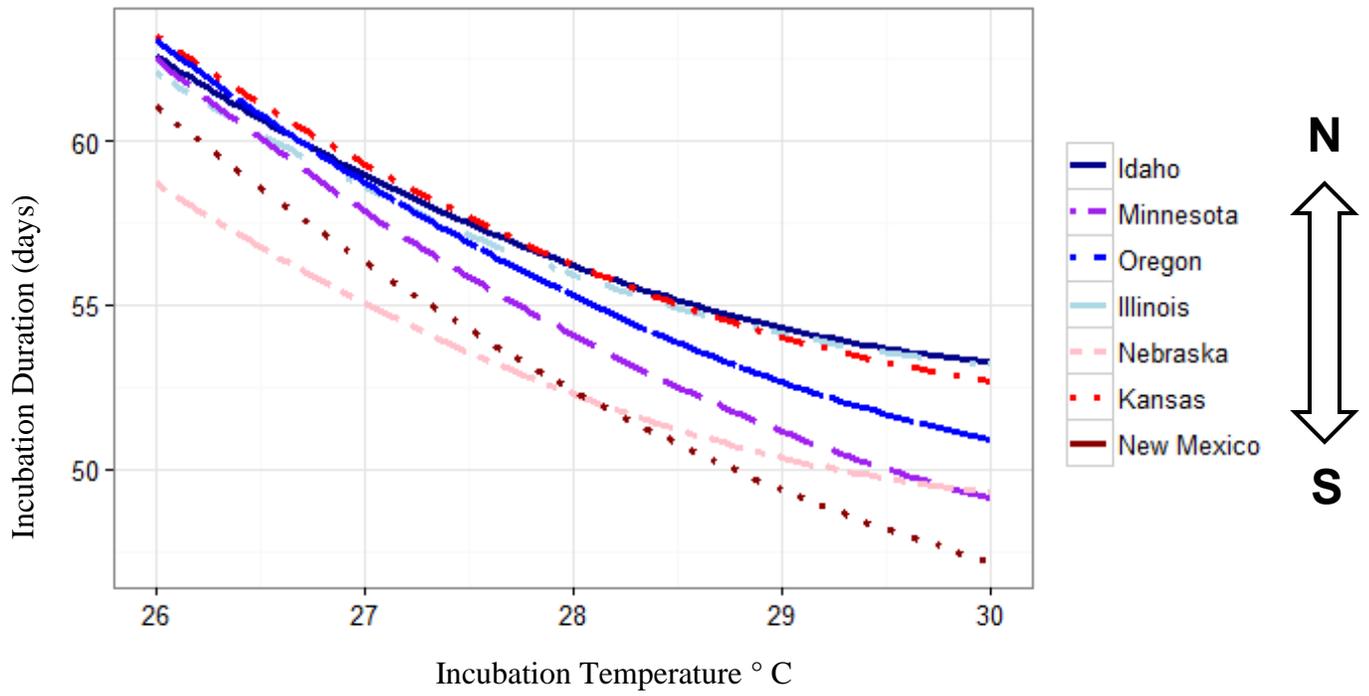


Figure 2: Model responses for developmental time (days) of painted turtle embryos from a given location and incubation temperature, with initial egg mass as a covariate.

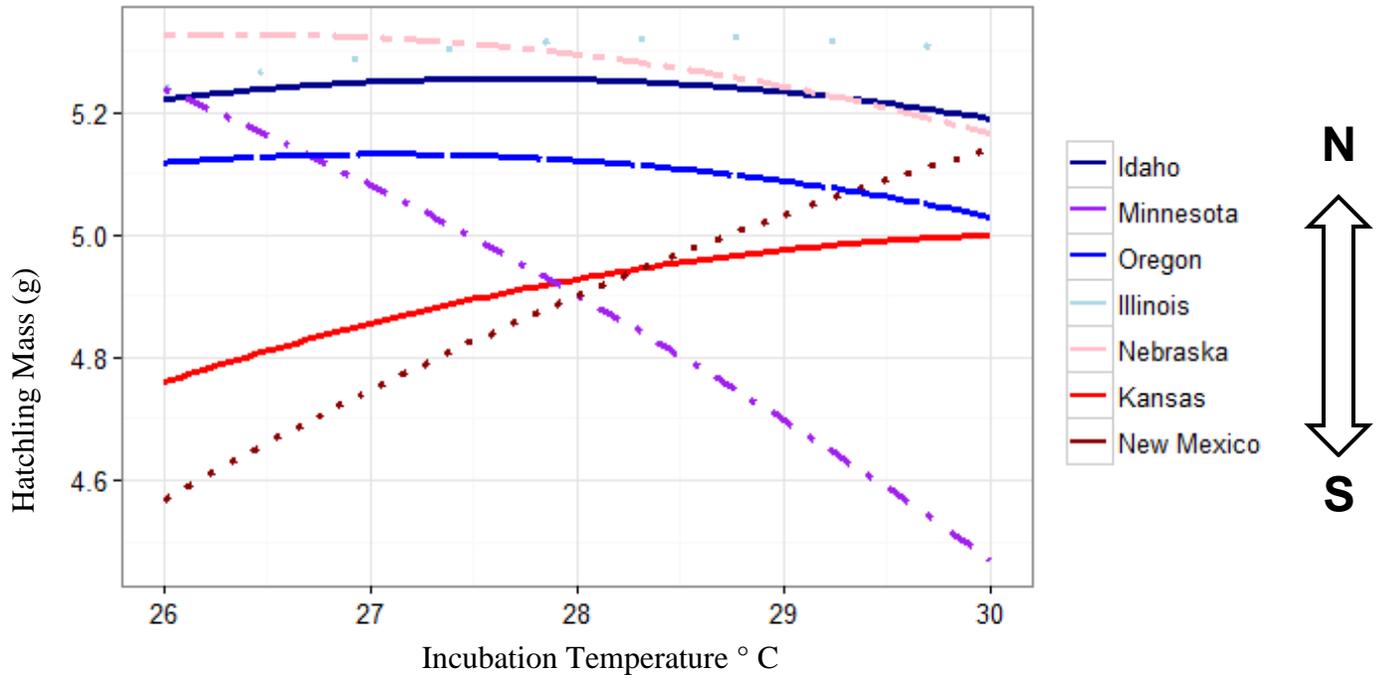


Figure 3: Model responses for mass (grams) of painted turtle hatchlings from a given location and incubation temperature, with initial egg mass as a covariate.

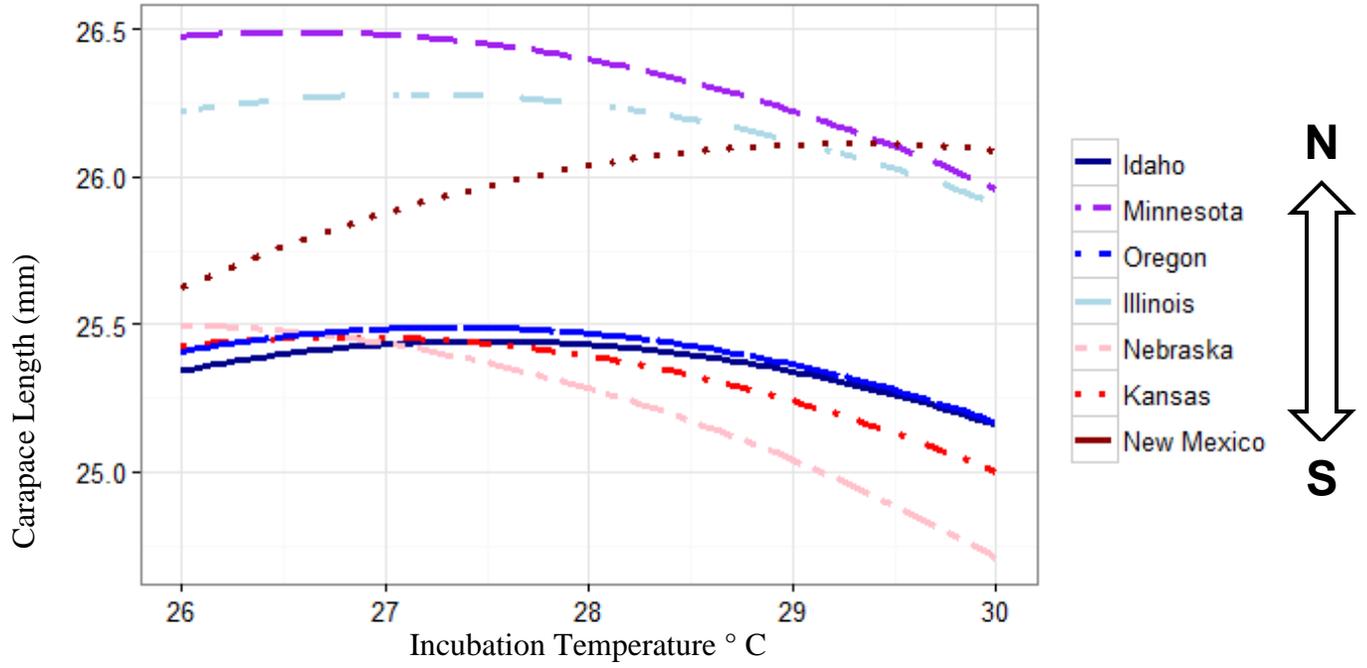


Figure 4: Model responses for carapace length (millimeters) of painted turtle hatchlings from a given location and incubation temperature, with initial egg mass as a covariate.

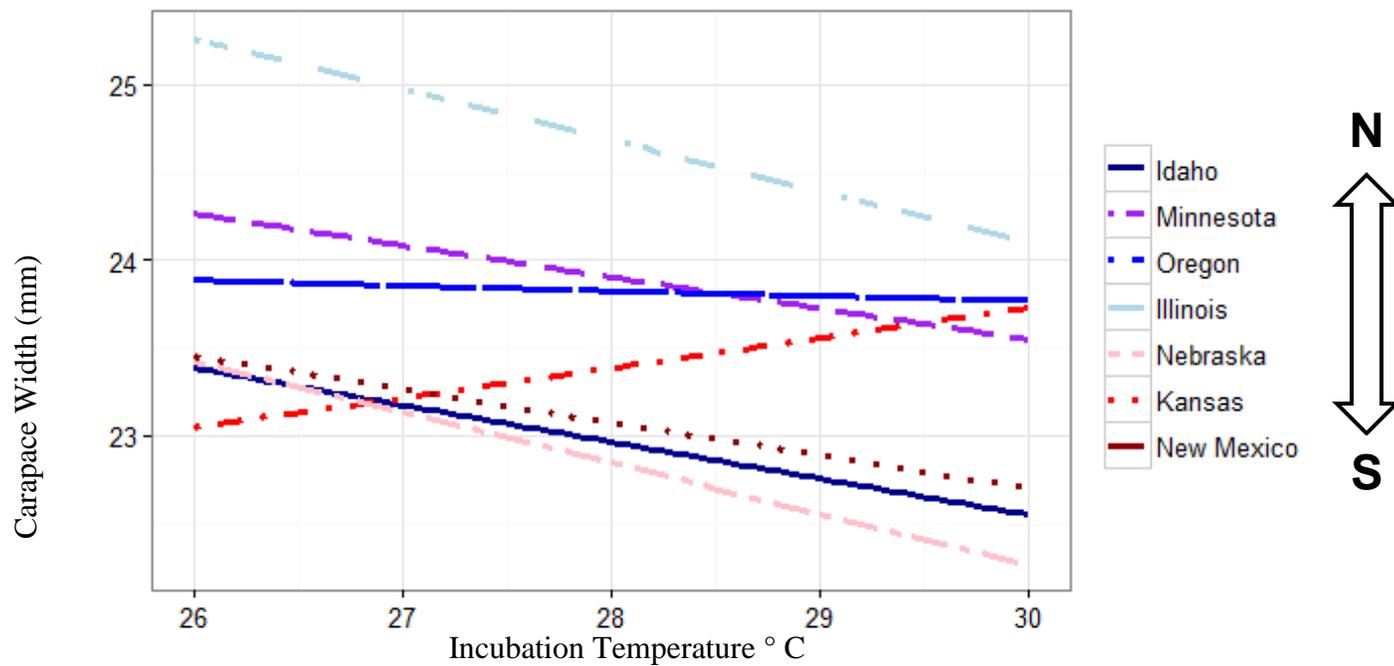


Figure 5: Model responses for carapace width (millimeters) of painted turtle hatchlings from a given location and incubation temperature, with initial egg mass as a covariate.

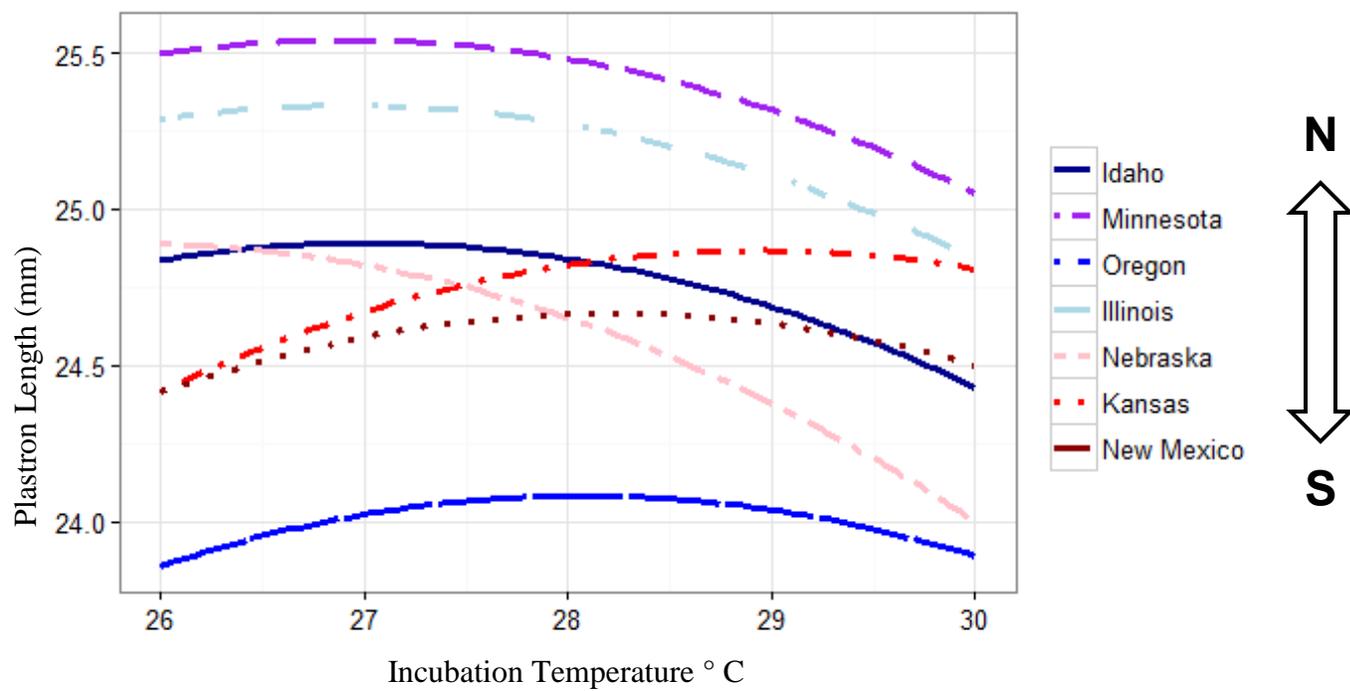


Figure 6: Model responses for plastron length (millimeters) of painted turtle hatchlings from a

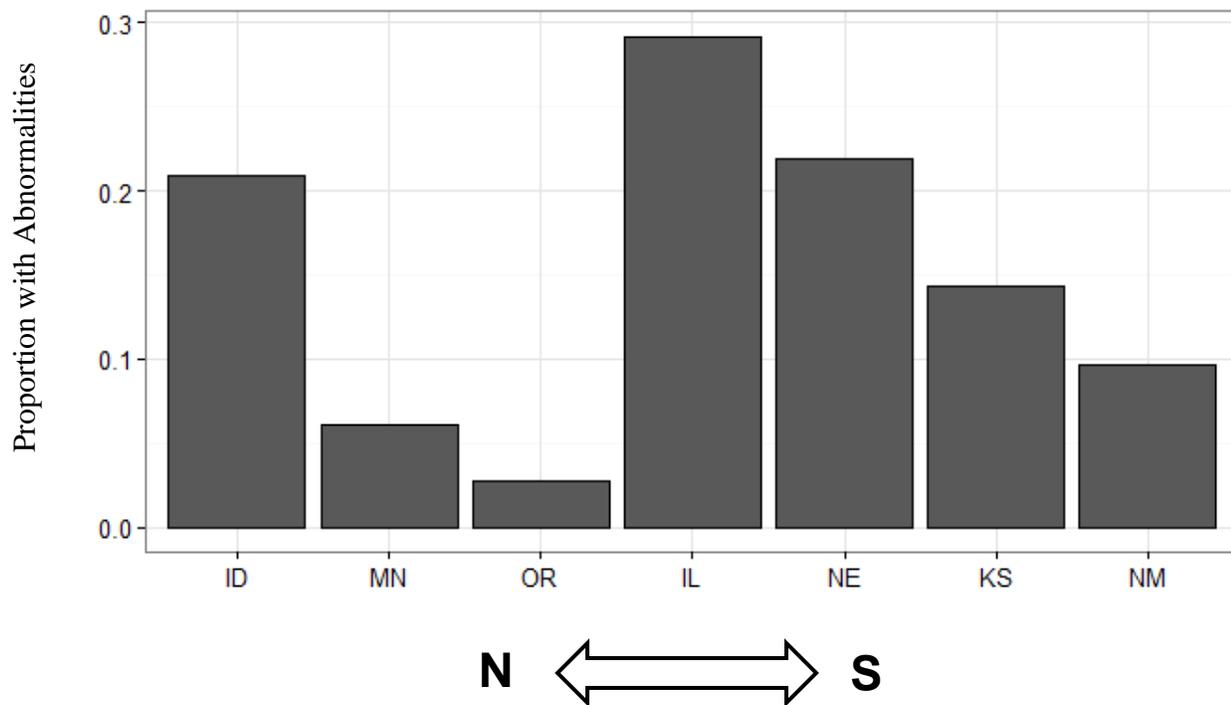


Figure 7: Proportion of painted turtle hatchlings with morphological abnormalities across locations.

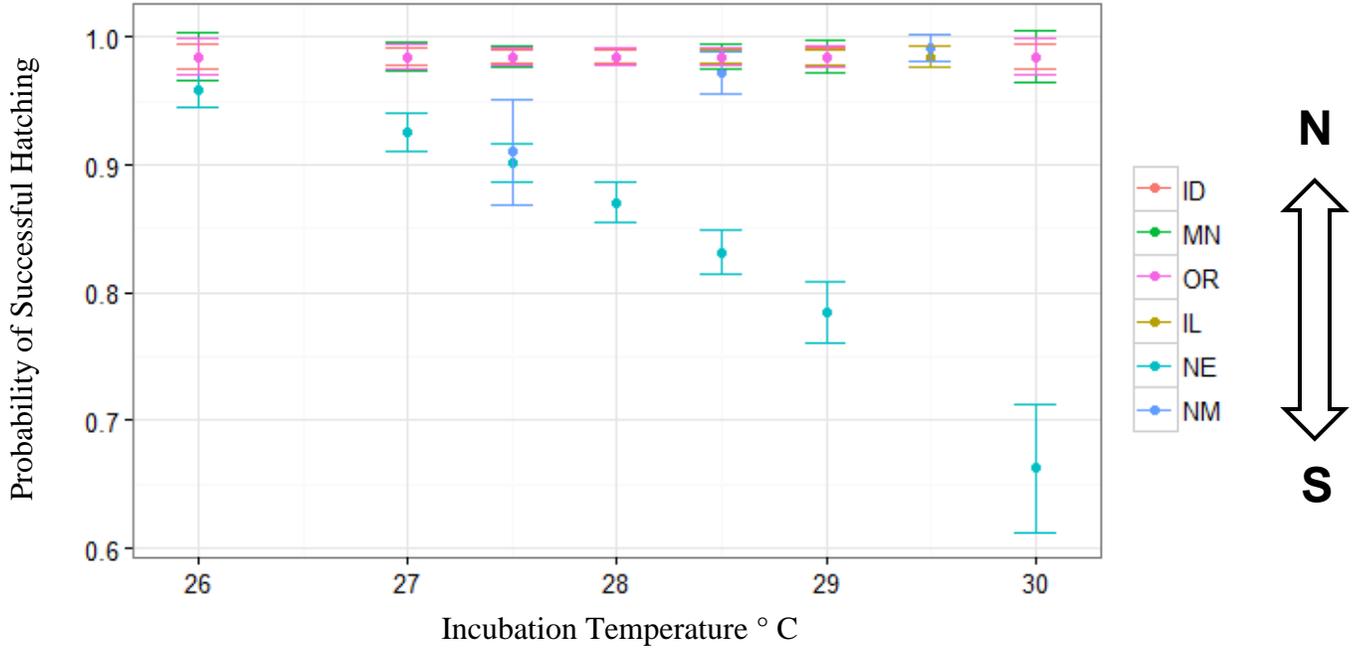


Figure 8: Least squares means and standard errors of the probability of painted turtle embryos successfully hatching across incubation temperatures for each location except Kansas (see text for details).

## CHAPTER 4

## SUMMARY AND CONCLUSIONS

The goal of this thesis was to elucidate how organisms with temperature-sensitive traits maintain viable populations across a vast geographic range that encompasses a diversity of environmental conditions. Questions of interest to biologist for decades have revolved around how such species match their phenotypes to the environment in which they are living (Bradshaw 1965; Williams 1966; Scheiner 1993). For species to persist across disparate environmental conditions, they will exhibit some combination of local adaptation or phenotypic plasticity (Kawecki & Ebert 2004; Charmantier *et al.* 2008; Baumann & Conover 2011; Ballen, Shine & Olsson 2015; Scheiner 2016). Oviposition, or nest-site choice, is a maternal effect that has the potential to influence not only embryonic phenotypes and survival, but also maternal fitness and survival across oviparous taxa (Mitchell 1975; Resetarits & Wilbur 1989; Li & Martin 1991; Resetarits 1996; Takegaki 2001; Brown & Shine 2004). Nest-site choice is especially pertinent in species with key life-history traits intrinsically tied to temperature that are at the mercy of environmental conditions during development. Thus, such maternal effects can play a central role in both adaptive and plastic responses to selection induced by local abiotic and biotic factors.

The scale, temporal and spatial, of this thesis work is rare. Evaluating these hard-won data helped us elucidate patterns associated with geographic variation in traits in a non-migratory terrestrial ectotherm, taking the first steps towards understanding the mechanism(s) by which these traits are maintained. Previous investigations of geographic variation in phenotypes have gleaned insights into how organisms are compensating for different environments experienced across a wide geographic range (Berven & Gill 1983; Niewiarowski & Roosenburg 1993; Li, Suzuki & Hara 1998; Foster & Endler 1999; Angilletta 2001; Armbruster *et al.* 2001; Züst *et al.* 2012; Duffy, Hice & Conover 2015). Few studies have investigated geographic variation in traits

of reptiles and nest-site choice at this spatial scale (Ewert, Lang & Nelson 2005; Doody *et al.* 2006a), and even fewer have characterized nest-site choice through multiple reproductive seasons at this substantial geographic scale (Weishampel *et al.* 2003). Our research contributions leveraged an integrative approach in which lab and field studies aided understanding how these organisms accommodate different thermal conditions during development.

First, nest-site choice appears to influence the thermal environments experienced by developing embryos. In particular, nest-site choice in painted turtles may mitigate the impacts of geographically-divergent ambient temperatures on incubating eggs. The microhabitats – measures of canopy cover and nest thermal environments – we measured differed among widely spaced turtle populations, with females choosing nest sites that generally had less canopy cover and higher temperatures compared to representative sites. Although locations differed in external thermal environments, natural nests nonetheless kept a tight range of mean temperatures differing by  $<3^{\circ}\text{C}$  across locations spanning nearly  $14^{\circ}$  latitude. This finding indicates females are buffering local climates to target a relatively small range of temperatures to which developing embryos are exposed. Moreover, the distributions of the thermal data from natural nests and representative sites indicate that, historically, females have chosen nest sites for embryos to avoid exposure to developmental thermal minima rather than maxima. Thus, based on our findings, it appears plasticity in nesting behavior for buffering developmental thermal maxima has not been under selection, as opposed to selection to supersede developmental thermal minima, which is problematic since climate is warming, not cooling.

Second, these painted turtle populations revealed local adaptation or pre-ovulatory maternal effects in phenotypic responses to incubation temperatures under common-garden conditions. Thermal reaction norms for developmental rate, hatchling mass, carapace width, and survival to

hatching varied among study locations. However, latitude and the 30-year mean July air temperature (i.e., during the middle of development) for each location were not strong predictors of these geographic differences. Consequently, common proxies, such as latitude, used to represent macrohabitat geographic patterns may not be reliable predictors of phenotypic variation in all cases. Thus, complex interactions between abiotic and biotic factors likely drive among-population phenotypic variation in this system. A tension between explanations of local adaptation and phenotypic plasticity for other traits in turtles is evident, but suggests that they coexist and are even potentially synergistic in natural populations (Packard & Packard 2000; Schwanz & Janzen 2008; McGaugh *et al.* 2009; Refsnider & Janzen 2012).

Supplementing the literature of how widespread organisms currently cope with divergent climatic conditions can aid in better predicting how they may accommodate future environmental changes. Of major concern is the rate at which global climate is currently changing and predicted to continue this century (IPCC 2014). These populations of *C. picta* have had thousands of years to locally adjust to differing climates post-Pleistocene glaciation (Starkey *et al.* 2003). Thus, many long-lived organisms may not be able to keep pace with the rapidity of the current environmental changes (Visser 2008). Long-lived organisms such as turtles might instead be more prone to adjust to local conditions, at least initially, through phenotypic plasticity because of their long generation times relative to the rate at which local conditions change. Hence, it is critical to understand if nest-site choice is a viable strategy for such organisms to keep pace with a warming climate (Refsnider and Janzen 2016).

To build off these findings, future research should examine the adaptive potential of nest-site choice. This adaptive potential could be investigated by determining at a finer scale how nonrandom nest-site choice is maintained across the species range, whether that be by allele

changes, plasticity, or a combination of genetic and plastic responses. Investigating GxE interactions in these populations, especially in Nebraska, could better elucidate how these populations are matching phenotypes, particularly hatchling mass, carapace width, and plastron length, to their environment. Given the findings of this thesis, additional experimental and modeling work should be done in the context of developmental and behavioral phenotypes and projected increasing climatic temperatures. Future experiments exposing *C. picta* embryos to increased developmental temperatures to match estimates of predicted climate warming would elucidate the influence potential climate changes could have on embryonic survival, phenotypes, and the potential for these abiotic conditions to affect demographic changes in the future. Additionally, the plethora of microhabitat data collected in this thesis can be used to better inform modeling efforts and assist in projecting into the future how the environments these populations are currently inhabiting are predicted to change.

This thesis highlights the importance of environmental effects during development influencing offspring phenotypes in *C. picta*, and the consequences of maternal effects, especially nest-site choice, in influencing such embryonic developmental conditions across a large spatial scale through time. Elucidating the responses to current divergent thermal conditions experienced among geographically widespread populations of organisms with temperature-sensitive traits could afford researchers a greater understanding of microevolutionary responses to environmental change. Additionally, identifying current organismal strategies for coping with a wide range of thermal conditions could aid our ability to predict population responses to future climate change, particularly globally increasing temperatures.

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