



Symposium Article

Temperature-Dependent Sex Determination under Rapid Anthropogenic Environmental Change: Evolution at a Turtle's Pace?

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Received February 15, 2015; First decision March 20, 2015; Accepted July 2, 2015.

Corresponding Editor: Robin Waples

Abstract

Organisms become adapted to their environment by evolving through natural selection, a process that generally transpires over many generations. Currently, anthropogenically driven environmental changes are occurring orders of magnitude faster than they did prior to human influence, which could potentially outpace the ability of some organisms to adapt. Here, we focus on traits associated with temperature-dependent sex determination (TSD), a classic polyphenism, in a model turtle species to address the evolutionary potential of species with TSD to respond to rapid climate change. We show, first, that sex-ratio outcomes in species with TSD are sensitive to climatic variation. We then identify the evolutionary potential, in terms of heritability, of TSD and quantify the evolutionary potential of 3 key traits involved in TSD: pivotal temperature, maternal nest-site choice, and nesting phenology. We find that these traits display different patterns of adaptive potential: pivotal temperature exhibits moderate heritable variation, whereas nest-site choice and nesting phenology, with considerable phenotypic plasticity, have only modest evolutionary potential to alter sex ratios. Therefore, the most likely response of species with TSD to anthropogenically induced climate change may be a combination of microevolution in thermal sensitivity of the sex-determining pathway and of plasticity in maternal nesting behavior.

Subject areas: Quantitative genetics and Mendelian inheritance

Key words: climate change, heritability, nest-site choice, phenology, pivotal temperature, reptile

Evolution by natural selection is a key mechanism by which organisms adapt to their environment (Darwin 1859). Adaptive changes in allele frequency of heritable traits, in response to selective pressures, allow organisms to keep pace with changes in the environment. Such microevolutionary responses permit organisms to remain adapted to their environment even when current conditions differ from those experienced by their ancestors (e.g., Aitken et al. 2008). The pace at which evolution occurs has been the subject

of intense study, and depends on several factors, including: 1) the strength of selection pressure acting on a trait of interest (Falconer and Mackay 1996); 2) the quantitative genetic underpinnings of a trait of interest (e.g., Falconer and Mackay 1996; Willi and Hoffmann 2008); and 3) the generation time of a given species, with short generation times generally resulting in faster rates of evolution than long generation times (e.g., Dunham and Overall 1994).

A hallmark of the “Anthropocene epoch” is that environmental changes are occurring orders of magnitude faster than they did prior to human influence. In particular, the global climate has warmed by 0.85 °C since the Industrial Revolution (IPCC 2013). Over the same time period, the oceans have become 26% more acidic due to oceanic uptake of CO₂ (IPCC 2013), while 33–50% of the Earth’s land surface has been altered by humans (Vitousek et al. 1997). The speed at which anthropogenic environmental changes are occurring may outpace many organisms’ ability to adapt to such changes through evolutionary responses (Aitken et al. 2008; reviewed in Lavergne et al. 2010). Species that fail to adapt to the novel environmental conditions imposed by human activity may face extinction, and we as humans will be faced with a loss of biodiversity and the ecosystem services provided by that biodiversity.

The focus of research on anthropogenic environmental change has begun to shift from compilations of the direct effects of rapid environmental change on organisms (e.g., Beebe 1995; Pike et al. 2006; Dunn et al. 2011) to a more integrative approach that seeks to understand how organisms might respond to rapid environmental changes (e.g., Hoffmann and Sgrò 2011; Walters et al. 2012; Kingsolver et al. 2013). In particular, scientists are increasingly interested in assessing the evolutionary potential of organisms in the context of rapid environmental change (Visser 2008; Lavergne et al. 2010; Chevin 2013). For example, what traits might be most affected by anthropogenic environmental change? Can the most-impacted traits evolve in response to anthropogenic environmental change and, if so, can they respond quickly enough to keep pace with the rapid rate of such change? If we know whether and how specific traits will evolve in response to rapid environmental changes, we can target our conservation efforts in ways that enhance species’ natural ability to cope.

Environmental sex determination (ESD) is an ideal trait for assessing the evolutionary potential of organisms to respond to rapid environmental change. In species with ESD, an individual’s sex is directly controlled by an environmental factor such as temperature or photoperiod, which produces the binary response of male or female. Unlike genotypic sex determination, in which sex is determined at fertilization by the complement of sex chromosomes present in the zygote, no sex chromosomes are present in species with ESD; instead sex is determined by some environmental cue during a discrete period later in embryonic development (Wibbels et al. 1994). Thus, in ESD systems a key demographic trait (i.e., sex ratio) is directly controlled by a single environmental parameter, which itself can vary widely in space and time and may be strongly influenced by anthropogenic activity.

ESD occurs in a diverse range of taxa, with an impressive diversity in the environmental cues that determine sex. Photoperiod determines sex in the saltbush *Atriplex halimus* (Talamali et al. 2003) and the amphipod *Gammarus duebeni* (McCabe and Dunn 1997). In the nematode *Mermis nigrescens*, sex is determined by the size of the grasshopper host in which an individual nematode develops (Craig and Webster 1982); in the echiurid worm *Bonellia viridis*, sex is determined by whether a larva settles on the seafloor or on a female conspecific (Baltzer 1914). The most widespread mode of ESD is temperature-dependent sex determination (TSD), in which the sex of offspring is determined by temperatures experienced by the developing embryo during egg incubation (Bull 1983). TSD occurs in some fish (e.g., *Menidia menidia*; Conover and Kynard 1981) as well as in many reptiles, including all crocodilians (Lang and Andrews 1994); the tuatara, sole member of Order Rhynchocephalia (Cree et al. 1995); nearly all turtles (Janzen and Krenz 2004); and some lizards (Bull 1983; Janzen and Paukstis 1991).

Within TSD, several different patterns of sex determination are present. In species with Type 1a TSD, including most turtles such as red-eared sliders (*Trachemys scripta*), females are produced at high incubation temperatures and males at low temperatures (e.g., Ewert et al. 1994). Type 1b TSD, which occurs in the tuatara (*Sphenodon punctatus*), demonstrates the reverse pattern, with males produced at high incubation temperatures and females at low temperatures (e.g., Cree et al. 1995). Finally, a few species including the snapping turtle (*Chelydra serpentina*), crocodilians (e.g., *Alligator mississippiensis*), and lizards (e.g., *Amphibolurus muricatus*) have Type 2 TSD, where males are produced at intermediate incubation temperatures and either females or a mixed sex ratio are produced at extreme (either high or low) temperatures (Ewert et al. 1994; Lang and Andrews 1994; Harlow and Taylor 2000). The thermosensitive period, during which sex determination occurs, is generally the middle third of embryonic development (Wibbels et al. 1994). Sex determination is affected by both mean incubation temperature as well as the magnitude of daily temperature fluctuation (Paitz et al. 2010; Warner and Shine 2011). There is also mounting evidence that the effect of temperature fluctuation on hatchling phenotype, including sex, differs depending on whether such fluctuation occurs primarily around the upper or lower unisexual mean temperature (Les et al. 2009; Neuwald and Valenzuela 2011). Laboratory incubation experiments designed to test the effects of temperature on offspring sex ratio have traditionally been conducted using constant incubation temperatures, which may not accurately replicate the incubation conditions in natural nests that experience daily and seasonal fluctuations. To translate results from constant-temperature experiments into projections about sex determination under more variable field conditions, constant temperature equivalent (CTE) models have been developed to condense variation in natural nests into a single number (the CTE), which can be used to predict sex ratios in species with TSD (Georges 1989). Recently, CTE models have been modified to account for interactions among climate change, nesting phenology, and seasonal thermal pattern on temperature during the thermosensitive period and thus on offspring sex ratio (Telemece et al. 2013).

Sex ratio in species with TSD is extremely sensitive to temperature (Janzen 1994a; Chu et al. 2008; Mitchell et al. 2008). Regardless of the type of TSD exhibited by a species, the transitional range of constant incubation temperatures, within which a mixed sex ratio is produced, is narrow (often only 1–2 °C; Hulin et al. 2009). Therefore, small changes in environmental temperatures could result in substantial skews in sex ratios, which could lead to population declines and extinctions if species with TSD are unable to adjust to a changing climate. Turtles are an excellent model system for studying the evolutionary potential of ESD, as most species have TSD and are conceivably vulnerable to the effects of climate change. Moreover, because turtles comprise one of the most endangered major groups of animals in the world (IUCN 2011; Van Dijk et al. 2014), understanding the evolutionary potential of TSD has critical conservation implications.

In this review, we focus on understanding the evolutionary potential of TSD in the context of anthropogenic climate change, drawing from our long-term integrative studies in a model turtle species. Tackling this question presents empirical challenges, which we address below. First, we demonstrate that TSD is, in fact, sensitive to climatic variation in terms of sex-ratio outcomes. Second, given that TSD is sensitive to climatic variation, we identify the heritability of TSD and quantify the evolutionary potential of specific traits involved in TSD. Several major factors affect sex-ratio dynamics in

species with TSD, including 1) the sensitivity of the sex-determining pathway to temperature, 2) maternal nest-site choice, and 3) nesting phenology (Bulmer and Bull 1982). Below we discuss the evolutionary potential of these 3 factors and their likely contribution to the persistence of turtles with TSD in the face of anthropogenic climate change. We also briefly summarize the evidence supporting phenotypic plasticity—the expression of different phenotypes (either behavioral or physiological) under different environmental conditions—as a mechanism that may allow species with TSD to keep pace with anthropogenically driven environmental change. Finally, we discuss how the results of our integrative research program on turtles can be used in predicting whether and how other taxa with ESD may evolve in response to rapid environmental changes.

Field Methods

Over the past several decades, our research program has focused on integrating field and laboratory studies to evaluate population dynamics and species persistence in the context of anthropogenically driven climate change (e.g., Harms et al. 2005; Schwanz et al. 2009, 2010a). Much of our research has been conducted using the painted turtle (*Chrysemys picta*), a model freshwater turtle species with Type 1a TSD. Painted turtles are a common wetland species occurring throughout much of North America. They spend most of the year in a variety of aquatic habitats, hibernating at the bottom of wetlands during the winter and becoming active again during early spring. In May and June, females come ashore to construct shallow (5–10 cm deep) nests in open habitats such as beaches and lawns. Eggs incubate within the nest cavity throughout the summer, with the month of July roughly corresponding to the thermosensitive period during which sex determination occurs. Offspring hatch in August or September, but in most populations hatchlings remain within the nest cavity throughout their first winter and emerge the following spring, at which time they travel from their nest site to wetland habitat (Gibbons 2013). In our study population, females become sexually mature at 6–7 years of age, while males mature at 3–4 years of age (Bowden et al. 2004; Schwanz et al. 2010a). Once reaching maturity, females breed nearly every year and construct 1–3 nests annually. Lifespan in our study population is unknown, but is probably substantially less than the maximum lifespan of ~50 years reported for other populations (e.g., Congdon et al. 2003).

We have studied painted turtles at a nesting beach on an island in the Mississippi River, IL, USA since 1988. The nesting beach is situated on the east side of the island, which is adjacent to a shallow backwater slough from which hundreds of female painted turtles emerge each spring to nest. Each year, a team of researchers monitors the nesting beach hourly from mid-May through early July for nesting painted turtles (summarized in Refsnider et al. 2014). Nesting turtles are observed from a distance to prevent disturbance, and are captured by hand upon completion of nesting. All captured females are individually marked with a unique combination of notches filed into the marginal scutes of the carapace. Females are measured (carapace and plastron length and width), 1.5 mL of blood is collected from the caudal vein, and then females are released into the slough. We also quantify vegetation cover directly over each nest using a spherical densiometer with readings taken from each of the 4 cardinal directions (Janzen 1994b) or, more recently, using hemispherical photography (Doody et al. 2006b; Mitchell et al. 2013). Finally, we plot each nest's precise location using Cartesian coordinates established for each nest using the program INTERPNT (Boose et al. 1998). In September of each year, when offspring have hatched from

eggs but remain within the nest cavity, we retrieve hatchlings from all nests to quantify clutch sex ratios by macroscopic examination of the gonads as in Schwarzkopf and Brooks (1985).

Evaluating the Evolutionary Response of TSD to Rapid Environmental Change

Predicting the evolutionary response of organisms to a specific source of selection is an important goal of contemporary evolutionary biology. To achieve the goal of accurately predicting an evolutionary response requires, first, quantifying the degree to which a trait of interest is sensitive to a given selective pressure, and second, identifying the heritability of the trait of interest. Consequently, to evaluate the microevolutionary response of species with TSD to anthropogenic climate change requires determining whether TSD is sensitive to climatic variation (i.e., exhibits phenotypic variation), and then identifying the quantitative genetics of specific traits involved in TSD that could evolve in response to changing environments.

Are Populations with TSD Sensitive to Climatic Variation?

In systems where a key demographic parameter, such as sex ratio, is highly sensitive to a specific environmental variable, such as temperature, it seems intuitive that the population as a whole would be susceptible to variation in climate. Controlled laboratory studies demonstrate that sex ratios of species with TSD can be altered substantially by manipulating egg incubation temperatures (e.g., Bull et al. 1982; Janzen 1992; Ewert et al. 1994; Warner and Shine 2011). However, linking sex ratio and climatic variation in the wild requires long-term studies of individual populations. At our painted turtle study site in Illinois, the offspring cohort sex ratio is strongly correlated with climate, with male-biased sex ratios produced in years with cooler July air temperatures and female-biased sex ratios produced in years with warmer July air temperatures (Janzen 1994a; Schwanz et al. 2010a; Figure 1). Importantly, the effects of climatic variation are not limited to the sex ratio of annual cohorts. Indeed, variation in sex ratios resulting from year-to-year climatic differences carries through to the adult population: adult female recruitment into our Illinois population is correlated with the number of female offspring recruited 6 years earlier (Schwanz et al. 2010a). Moreover, comparisons of wild painted turtle populations across a latitudinal and climatic gradient demonstrate that nesting turtles match the thermal incubation conditions of their nests with the local climate by altering thermally sensitive characteristics of their nest sites (Refsnider et al. 2014). This matching of incubation conditions to local climatic conditions results in similar mean nest temperatures across the geographic range of the species (Refsnider et al. 2014). Together, these lines of evidence demonstrate that turtle populations with TSD are highly sensitive to climatic variation.

What is the Evolutionary Potential of TSD in the Wild?

Having demonstrated that populations with TSD are sensitive to climatic variation (Figure 1), we now must identify specific traits involved in TSD that could potentially evolve in response to environmental variation such as anthropogenic climate warming. In reptiles with TSD, the major traits that contribute to a female's offspring sex ratio appear to be: 1) pivotal temperature, or T_{piv} [the constant incubation temperature at which offspring sex ratio shifts from predominantly one sex to the other, and which is used as a measure of the thermal sensitivity of the sex-determining pathway (Georges et al. 1994; Giron-dot 1999)]; 2) maternal nest-site choice (characteristics of the nest site such as shade cover, soil moisture

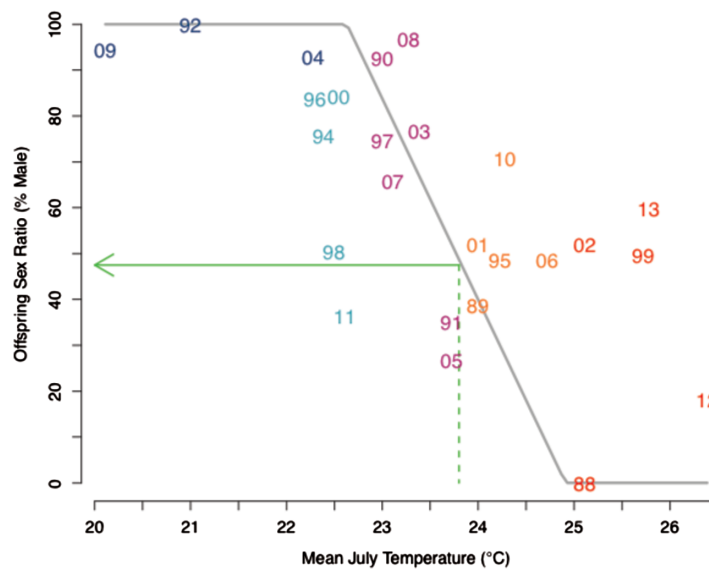


Figure 1. Annual cohort sex ratio (% male) of hatchling painted turtles (*Chrysemys picta*) at an Illinois study site from 1988 to 2014 as a function of July air temperature (modified from Figure 1 in Janzen 1994a and from Figure 1B in Schwanz et al. 2010a). The dashed line is the 76-year grand mean July air temperature at Fulton Dam, 6.5 km south of the field site; the green arrow indicates the annual cohort sex ratio predicted by this temperature. No sex ratio data are available for 1993 and 2014 (summer floods eliminated recruitment in those years). Cooler years (bluer numbers) yield more male offspring and warmer years (redder numbers) yield more female offspring.

content, nest depth, etc. that are selected by the nesting female); and 3) nesting phenology (the seasonal timing of nest construction). To determine the extent to which any of these traits could contribute to the microevolution of TSD under anthropogenic climate change, at a minimum we must quantify the heritability of these traits in wild populations.

Pivotal temperature (T_{piv})

Reptiles with TSD have survived numerous periods of global warming and cooling in the past, and evolution of the thermal sensitivity of the sex-determining pathway has been proposed as a likely mechanism for preventing severe sex-ratio skews and subsequent extinctions resulting from such climatic changes (Bulmer and Bull 1982). Although T_{piv} is generally considered to be a population-level parameter, individual females within a population exhibit variation in terms of their offspring's sensitivity to incubation temperatures (Conover and Kynard 1981; Bull et al. 1982; Janzen 1992; Rhen et al. 2011). Heritability of T_{piv} measured in our Illinois painted turtle population in the wild is 0.35, which is high enough to permit some evolution of this trait (McGaugh et al. 2011). Indeed, wild populations of painted turtles exhibit small differences in T_{piv} across the species' geographic range (Morjan 2003; Refsnider et al. 2014), suggesting that T_{piv} likely permits some degree of local adaptation to prevailing climate. However, the differences in T_{piv} between populations appear to be insufficient to entirely compensate for climatic differences between those populations (Morjan 2003). Furthermore, because many nests are so warm or cool as to override genetic effects (Janzen 1994b), heritable variation in T_{piv} available to support an evolutionary response to selection is actually much lower (Janzen 1994a). For example, while field-measured heritability of T_{piv} in the Illinois population is 0.35, the heritability for purposes of predicting evolutionary response to selection is estimated to be 0.13 (McGaugh et al. 2011). Therefore, microevolution of T_{piv} alone is unlikely to entirely keep pace with the rapid rate of anthropogenic climate change.

Nest-site choice

In oviparous species, such as most reptiles, females can exert some control over the incubation conditions experienced by developing embryos through their selection of a nest site and how they construct their nest (reviewed in Refsnider and Janzen 2010). In painted turtles, nest sites under greater amounts of shade cover are cooler, and produce a higher proportion of males, than more open nest sites; therefore, shade cover may be a reliable cue nesting females can use to predict the sex ratio a given nest site is likely to produce (Morjan and Janzen 2003). Although individual females demonstrate consistent year-to-year preferences for nest sites with specific amounts of shade cover (Janzen and Morjan 2001; Kamel and Mrosovsky 2006), field estimates of heritability for nest-site choice in terms of shade cover are only 0.04 across years for painted turtles at our Illinois site (McGaugh et al. 2010). Such low heritability for choice of shade cover over nest sites likely constrains the microevolution of maternal nest-site choice based on shade cover. Intriguingly, though, the inheritance of this trait exhibits context dependence such that heritability is 0.19 after hot winters (McGaugh et al. 2010). Thus, nest-site choice under a global warming scenario conceivably could respond evolutionarily to some extent, although this behavior is expressed only by females. Hence, as with T_{piv} , microevolution of female choice of shade cover over nest sites by itself would appear to be implausible to compensate for rapid changes in environmental conditions.

Nesting phenology

Phenology, or the timing of specific life-history events, is critical for matching an organism's ecology to prevailing environmental conditions. Anthropogenic climate change is disrupting phenology in a wide variety of taxa (reviewed in Parmesan 2006) and will likely lead to mismatches between organisms and their environments (e.g., Crozier et al. 2011; Dunn et al. 2011). Advances in the breeding season, concomitant with a warming climate, have been observed in mammals (Berteaux et al. 2004), birds (Crick et al. 1997; Gill et al.

2014), frogs (Beebe 1995; Todd et al. 2011), angiosperms (Fitter and Fitter 2002; Ellwood et al. 2013), and numerous invertebrates, suggesting that many species can adjust phenology in response to a changing climate. However, whether such phenological changes are based on microevolutionary responses or behavioral plasticity remains an open question. In red squirrels, both microevolutionary responses and phenotypic plasticity have allowed Arctic populations to keep pace with a warming climate over a 10-year period (Berteaux et al. 2004). In contrast, short-term changes in the breeding phenology of frogs were attributed to physiological plasticity rather than adaptive evolution in response to a changing environment (Beebe 1995). Breeding season advances have also been observed in turtles, including red-eared sliders (Tucker et al. 2008). In painted turtles at our Illinois site, the timing of the nesting season is correlated with the previous winter's climate (in terms of heating degree-days; Schwanz and Janzen 2008), but field heritability for timing of nesting was negligible across years (McGaugh et al. 2010). Yet, like nest-site choice, onset of the nesting season had a higher heritability (0.17) after hot winters. Importantly, however, modeling studies suggest that even if female painted turtles were able to advance nesting phenology sufficiently to continue nesting at the same soil temperature every year, the increase in soil temperatures later in incubation would be lethally high (Telemeo et al. 2013). Therefore, advances in breeding phenology could result in increased embryo mortality, which would select against changes in nesting phenology.

The Role of Plasticity as a Response to Rapid Climate Change in Species with TSD

In general, little evidence has been found to suggest that long-lived organisms are able to evolve rapidly enough to keep pace with anthropogenic climate change (reviewed in Hoffmann and Sgrò 2011; Urban et al. 2014). Our results from a 27-year ongoing study of painted turtles support this idea, in that major traits underlying sex-ratio responses to climatic variation exhibit only modest, if any, heritability in the field (McGaugh et al. 2010, 2011). What other mechanism(s) might allow long-lived species with TSD to keep pace with rapid environmental changes? One possibility is essentially the flip side to heritable variation: phenotypic plasticity. In principle, plasticity in sex-ratio traits in species with TSD could compensate for short-term changes in environmental conditions, allowing evolution time to “catch up” to the novel climate (Morjan 2003; Huey and Tewksbury 2009; Urban et al. 2014).

Physiological plasticity (e.g., acclimation), especially in response to temperature, is a core feature of ectotherms like turtles (Seebacher et al. 2015). While this mechanism could operate in species with TSD, evidence demonstrating its utility is sparse. Schwanz et al. (2010b) found that annual T_{piv} for our Illinois population of painted turtles was positively correlated with overall mean July nest temperature (Figure 2), thereby demonstrating plasticity in the potential to produce particular offspring sex ratios. Mechanistically, genetic or epigenetic parental effects could drive such an outcome. For example, in a laboratory study of T_{piv} , the ratio of estradiol to testosterone in yolks of painted turtle eggs at oviposition was correlated with offspring sex ratio (Bowden et al. 2000). Even so, the broader occurrence of this endocrinological mechanism or other such physiological parental effects in species with TSD is questionable (e.g., Rhen et al. 2011). Gene-by-environment interactions may also be important, as evidence from a lizard with TSD suggests that variation in offspring phenotypes, including sex ratio, has a significant genetic component (Warner et al. 2008). Other epigenetic factors (DNA

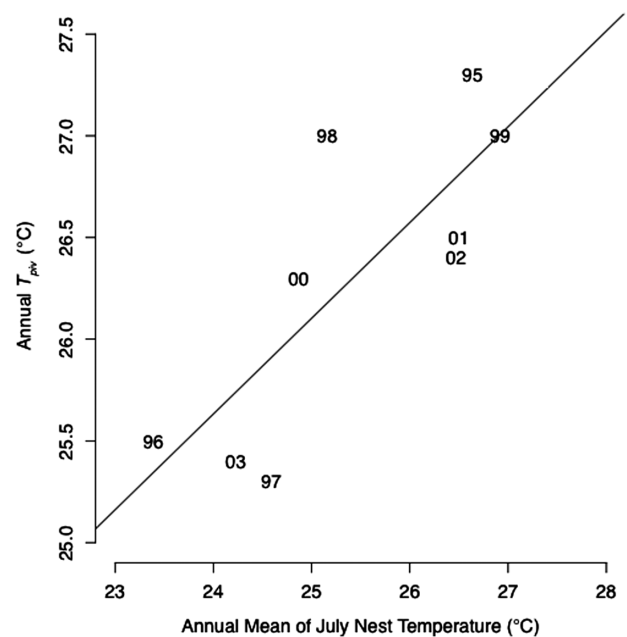


Figure 2. Annual T_{piv} is positively correlated with the annual mean July temperature in painted turtle (*Chrysemys picta*) nests at an Illinois study site ($r^2 = 0.62$, $P = 0.011$; modified from Figure 4 in Schwanz et al. 2010b). Additional years are not available because of negligible nest survival, extreme cohort sex ratio, and/or lack of nest temperature data. The regression line suggests that, at any given nest temperature (e.g., 26 °C), years (=2-digit numbers) with cooler nests have a higher probability of yielding some female offspring than years with warmer nests, implying a previously unsuspected level of physiological plasticity in T_{piv} .

methylation, histone modifications, etc.) are also worth exploring (Navarro-Martin et al. 2011; Warner et al. 2013) to explain our findings that physiological plasticity can result in the production of particular offspring sex ratios (Schwanz et al. 2010b; Figure 2).

Behavioral plasticity is exceedingly common in animals. With respect to TSD, plasticity in nest-site choice provides at least a partial buffer from the effects of climate change. In particular, female adjustment of nest depth (Doody et al. 2006a; Telemeo et al. 2009) and choice of overhead shade cover (Refsnider and Janzen 2012) can alter a nest's incubation temperature and thereby compensate for changes in climatic conditions. However, constraints may inhibit or prevent plasticity in nest-site choice from being expressed. For example, soil hardness limits the ability of female tuatara to construct deeper nesting burrows (Mitchell et al. 2008). In painted turtles, availability of suitable nesting habitat constrains females' choice of shade cover over potential nest sites along the species' equatorial range edge (Refsnider et al. 2013a). Furthermore, in some populations of painted turtles, rear limb length constrains ability to construct deeper, and therefore cooler and less variable, nests to compensate for a warming climate (Refsnider et al. 2013b). These results demonstrate that, while phenotypic plasticity can play an important role in allowing species with TSD to compensate for anthropogenic climate change, the ability of species to express such plasticity may be limited for a variety of reasons. Beyond these environmental and physical limits, it will also be important to ascertain whether these animals possess the capacity to assess current sex ratios or environmental conditions and adjust nesting behavior accordingly (e.g., Warner and Shine 2007).

As with nest-site choice, plasticity in nesting phenology is apparent in reptiles with TSD. The low repeatability in individual timing

of nesting across years (Schwanz and Janzen 2008) suggests that this behavior is not canalized or synchronized overall. Consequently, timing of nesting may make for a poor target for selection in this instance. Whereas nesting earlier may have some fitness advantage by increasing the odds of additional reproductive events in the same season (e.g., Tucker et al. 2008), as discussed above altered nesting phenology is unlikely to be the sole mechanism for adjusting to rapid environmental change, whether or not it is mostly heritable or mostly plastic (Telemeco et al. 2013).

Discussion

The rapid environmental changes currently being imposed on the world's ecosystems by human activities are likely to cause substantial changes in community composition, species interactions, and traits of individual species. Species that are unable to respond to anthropogenically induced environmental change, likely because of the unprecedented rate at which such environmental changes are occurring, may face extinction (Palumbi 2001). However, species may have the capacity to respond to anthropogenically induced environmental change, such as climate change, via several mechanisms that could prevent, or at least delay, species extinction. In particular, some species, especially those with short generation times and a high level of genetic variation supporting local adaptation to climate, may have sufficient adaptive potential to evolve in response to climate change (Bell and Collins 2008; Visser 2008; Lavergne et al. 2010). However, the speed at which climate change is currently occurring may preclude microevolutionary responses even under such favorable circumstances (Visser 2008; Lavergne et al. 2010).

Our long-term research on long-lived painted turtles, integrating laboratory and field studies in wild populations, has provided several key insights into the evolutionary potential of TSD-related traits to keep pace with anthropogenically driven climate change. First, the population demography of animals with TSD is extremely sensitive to climatic variation (Figure 1). Thus, the capacity for strong sex-ratio selection induced by altered thermal environments is present. Second, key traits associated with TSD exhibit different patterns of adaptive potential. In particular, T_{piv} displays moderate heritable variation, whereas nest-site choice and nesting phenology have very modest evolutionary potential to alter nest sex ratios and, even then, this heritability is condition-dependent. Therefore, if species with TSD are to respond to climate change through genetically based adaptation, microevolution of T_{piv} seems to be the most likely route by which such adaptation might occur. However, the magnitude of the field heritability of T_{piv} still may permit insufficient microevolution to allow species with TSD to entirely keep pace with a rapidly changing climate (Janzen 1994a; Mitchell and Janzen 2010).

How are Species with TSD Likely to Respond to Rapid Climate Change?

Based on existing evidence, how might turtles and other species with TSD respond to altered sex ratios, perhaps in combination with microevolution of T_{piv} ? Several possible pathways have been proposed by which reptiles with TSD may compensate for the effects of a rapidly changing climate (Huey and Janzen 2008). First, could the sex-determining mechanism itself shift, for example, from an environmentally determined system to a chromosomally determined one (genotypic sex determination, GSD)? Turnovers of sex-determining systems seem relatively frequent in fishes (Heule et al. 2014) and reptiles (Janzen and Krenz 2004; Pokorná and Kratochvíl 2009),

demonstrating the lability of sex-determining mechanisms in at least some taxa on an evolutionary time scale. However, transitions between sex-determining systems (such as ESD to GSD or vice versa) in animals occur through the recruitment of new master-switch genes that control sexual fate (Bachtrog et al. 2014), and there are likely limits on the types of genes that could be co-opted as master sex-determining genes (Graves and Peichel 2010), which could constrain ESD-to-GSD transitions in many taxa. Second, rather than evolution of the entire sex-determination pathway, could the thermal sensitivity of the pathway shift to track changing climatic conditions? Our estimates of heritability for thermal sensitivity of the sex-determination pathway in the field are significant. However, given a modest scenario of a 4 °C rise in global temperatures (IPCC 2013), T_{piv} presumably also would have to increase 4 °C to keep pace. With a relevant heritability estimate of 0.13 for T_{piv} , we can employ the breeder's equation (i.e., the evolutionary response is a product of the heritability and the strength of selection; $R = h^2 \times S$) to calculate that the strength of selection on T_{piv} would be 30.75. With an estimated standard deviation of 1.13 (Janzen 1994a), T_{piv} would have to increase by ~27 standard deviations by 2100 (~15 painted turtle generations). A response of this magnitude seems highly unlikely to permit evolutionary rescue (Gomulkiewicz and Shaw 2012), but perhaps any microevolutionary response in T_{piv} , combined with additional compensatory mechanisms, could allow species with TSD to keep pace with a rapidly changing climate.

What additional mechanisms, when combined with microevolutionary responses of the thermal sensitivity pathway, could allow species with TSD to keep pace with climate change? Some taxa, such as butterflies (Parmesan et al. 1999) and birds (Tingley et al. 2012), have shifted geographic ranges. Geographic range shifts may have played an important role in maintaining local adaptation in species with TSD over geological time, as evidenced by populations of such taxa currently occupying habitats that were covered by glaciers only 20 Kya (e.g., Holman 1995). Contemporary shifts in geographic range seem unlikely for most species with TSD, however, because reptiles are generally poor dispersers and cannot easily travel through inhospitable areas to reach new suitable habitat (reviewed in Mitchell and Janzen 2010). Assisted colonization, despite its controversial aspects, is a conceivable part of the solution for at least some taxa with TSD (e.g., Miller et al. 2012). Finally, nesting behavior may be adjustable in response to climatic variation. We have estimated low levels of heritability for nest-site choice in painted turtles (McGaugh et al. 2010), suggesting that microevolution of this trait is unlikely to occur rapidly enough to keep pace with anthropogenic climate change. However, behavioral plasticity in nest-site choice has been demonstrated in painted turtles (Refsnider and Janzen 2012) and other species with TSD (reviewed in Urban et al. 2014) to compensate for novel climatic conditions.

If the climate continues to warm, reptiles with TSD that fail to adjust through phenotypic plasticity or microevolution are likely to experience severe demographic consequences. Intuitively, we might expect that population sex ratios will become increasingly skewed toward the sex produced at warmer temperatures. Empirical and modeling studies, however, suggest a more complex result. If climate change leads to earlier nesting, altered temperatures during the thermosensitive period could skew populations even more toward the "warmer" sex, as in tuatara that produce one clutch in a season (Mitchell et al. 2008). Alternatively, earlier springs may allow other species to produce an additional clutch each year, which would experience a cooler thermosensitive period later in summer and thereby skew population sex ratios toward the "cooler" sex, as in the

red-eared slider (Tucker et al. 2008). Populations with skewed sex ratios will experience decreased genetic diversity as heterozygosity is lost, and this effect will be exacerbated in populations with more extreme skews in sex ratio, and with sex ratios biased toward males rather than females (Mitchell and Janzen 2010). Modeling studies suggest that, in the absence of phenotypic plasticity or microevolution, climate change could result both in offspring sex ratios that are made up entirely of the “warmer” sex (Chu et al. 2008), as well as substantial embryonic mortality and overall decreased recruitment, as many nests experience lethally warm temperatures (Hawkes et al. 2007; Telemeco et al. 2013). Thus, climate change could lead to decreased recruitment and population declines via both direct (i.e., increased embryo mortality) and indirect (i.e., loss of genetic diversity due to biased sex ratio) pathways.

Adaptive evolution and phenotypic plasticity can enable populations to persist when environments change (Gienapp et al. 2008; Reed et al. 2010). Comparative studies and experimental approaches that evaluate climatic and phenotypic variation across broad spatial scales provide important insights into how organisms adapt to environments (e.g., Refsnider and Janzen 2012; Refsnider et al. 2014). The results of our long-term research suggest that the most likely response of species with TSD to anthropogenically induced climate change may be a combination of microevolution in thermal sensitivity of the sex-determining pathway, and of behavioral plasticity in maternal nest-site choice. Species that fail to respond to rapid climate change in this way may face extinction as environmental conditions warm beyond their current climatic envelope.

Research Needs

Additional research is clearly required to address more complex mechanisms that may allow species with ESD to respond to rapid, anthropogenic environmental changes. In our view, the best guidance for conservation and management actions on behalf of these increasingly imperiled reptiles with TSD will probably derive from well-designed, long-term field studies with a microevolutionary framework. Fieldwork is logistically challenging and often requires years to collect sufficient data, but controlled laboratory studies alone may not be enough to fully understand the “extended” life history of reptiles with TSD in the context of a changing environment. Key areas for future research include:

- (1) Quantitative genetic estimates in the wild for key TSD traits are essentially limited to a single species at present (i.e., the painted turtle). This taxonomic constraint limits the confidence we can place in general predictions about the evolutionary capacity of species with TSD to respond to sex-ratio selection induced by changing thermal environments. To wit, turtles are long-lived, as are crocodilians and tuatara, but the latter 2 taxa have different modes of TSD than do most turtles. Moreover, lizards and fish with TSD are short-lived and generally differ in mode of TSD from most turtles. Consequently, although hard won over many years, our field estimates for the quantitative genetics of TSD traits in painted turtles may not translate to other taxa with TSD and therefore highlight the urgency with which such studies in these other taxa are needed. Shorter-lived species with TSD could make excellent systems for imposing artificial selection at various levels on key TSD traits such as T_{piv} and evaluating the response over time (sensu Conover et al. 1992).
- (2) We have identified important TSD traits and targeted them for evolutionary genetic analyses in painted turtles, but these

assessments are nonetheless incomplete. For example, T_{piv} is not the only physiological component of thermal sensitivity that could contribute to the microevolution of TSD. T_{piv} essentially comprises the intercept of the sex-ratio reaction norm, whereas the slope of this relationship (the transitional range of temperatures; Hulin et al. 2009) or even the overall curvature (i.e., the mode of TSD) could also be important. Indeed, slope and curvature generally contribute to the greatest among-population differences in reaction norms (Murren et al. 2014). Consequently, quantitative genetic studies of these components are highly desirable to more accurately forecast the microevolutionary potential of TSD.

- (3) Another important aspect of quantitative genetic models concerns interactions between the various traits involved. When absent, the heritability of each trait can then be examined independently to weigh its microevolutionary influence. When present, these covariances can generate evolutionary dynamics not predicted solely by focusing on the linear aspects of these models (Falconer and Mackay 1996). To this point, epistatic interactions and/or genetic covariances among traits (or across environments) involved in the thermal sensitivity of the sex-determining pathway and nesting behavior have not been estimated, but will be required to generate more confidence in our understanding of the evolutionary response of TSD to sex-ratio selection induced by climate change.
- (4) Similarly, phenotypic plasticity can play an important role in species' responses to climate change, although the extent to which plasticity can be expressed may be constrained by ecological and evolutionary factors. Importantly, plasticity itself is a trait subject to natural selection and evolutionary change, where the direction and degree of response to environmental change is genetically variable and responsive to selection (West-Eberhard 1989). Thus, another fruitful avenue of research would be to determine the selection intensity on, and the heritability of, phenotypic plasticity in the TSD traits identified here, especially since phenotypic plasticity is expected to play a greater evolutionary role in long-lived species relative to shorter-lived ones (Chevin et al. 2010).
- (5) Elucidating the evolutionary potential of TSD at a more mechanistic level would be enlightening as well. For example, our ongoing research is leveraging the recently sequenced painted turtle nuclear genome (Shaffer et al. 2013) to understand how population differences in gene expression affect local adaptation of TSD to climate across the broad geographic range of this species. Epigenetic effects, such as DNA methylation, histone modifications, or RNA transcription, may affect genes and developmental pathways across the genome differentially depending on the environmental conditions experienced by the fathers, mothers, and developing embryos. Such epigenetic effects could allow for acclimation (i.e., phenotypic plasticity) to prevailing environmental conditions in the absence of a “traditional” microevolutionary response.
- (6) We also require a better understanding both of how contemporary populations of widespread species with TSD persist under contrasting thermal conditions, and how more narrowly distributed or ecologically specialized taxa with TSD are configured to adapt to changing climates. In other words, how have conspecific populations with TSD currently occupying vastly different environments solved the sex-ratio problem? And how might a “generalist versus specialist” perspective on this issue play out evolutionarily in an Anthropocene ecological theater (sensu Hutchinson 1965)?

Funding

The National Science Foundation (DEB-9629529, DEB-0089680, DEB-064932, and DEB-1242510 to F.J.J., IBN-0212935 to F.J.J. and R. Bowden, and IOS-1257857 to F.J.J. and D. Warner) and National Science Foundation Postdoctoral Research Fellowship (award no. 1202725 to J.M.R.).

Acknowledgements

We are grateful to Robin Waples for the opportunity to participate in the AGA symposium and to contribute to this themed issue; the U.S. Army Corps of Engineers, U.S. Fish and Wildlife Service, Illinois DNR, and Iowa State University IACUC for permission and approval of our long-term turtle studies at the Illinois field site; L. Hoekstra for preparing the figures; and, perhaps most significantly, the Turtle Camp crews for their many years of crucial collection of field data.

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