

2019

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Keywords

environmental change, microevolution, nesting behavior, pivotal temperature, quantitative genetics, reptile

Disciplines

Behavior and Ethology | Ecology and Evolutionary Biology | Genetics | Population Biology

Comments

This is a manuscript of an article published as Janzen, Fredric J., David M. Delaney, Timothy S. Mitchell, and Daniel A. Warner. "Do covariances between maternal behavior and embryonic physiology drive sex-ratio evolution under environmental sex determination?." *Journal of Heredity* (2019). doi: [10.1093/jhered/esz021](https://doi.org/10.1093/jhered/esz021).

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Do covariances between maternal behavior and embryonic physiology drive sex-ratio evolution under environmental sex determination?

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Abstract

Fisherian sex-ratio theory predicts sexual species should have a balanced primary sex ratio. However, organisms with environmental sex determination (ESD) are particularly vulnerable to experiencing skewed sex ratios when environmental conditions vary. Theoretical work has modeled sex-ratio dynamics for animals with ESD with regard to two traits predicted to be responsive to sex-ratio selection: (1) maternal oviposition behavior and (2) sensitivity of embryonic sex determination to environmental conditions, and much research has since focused on how these traits influence offspring sex ratios. However, relatively few studies have provided estimates of univariate quantitative genetic parameters for these two traits, and the existence of phenotypic or genetic covariances among these traits has not been assessed. Here, we leverage studies on three species of reptiles (two turtle species and a lizard) with temperature-dependent sex determination (TSD) to assess phenotypic covariances between measures of maternal oviposition behavior and thermal sensitivity of the sex-determining pathway. These studies quantified maternal behaviors that relate to nest temperature and sex ratio of offspring incubated under controlled conditions. A positive covariance between these traits would enhance the efficiency of sex-ratio selection when primary sex ratio is unbalanced. However, we detected no such covariance between measures of these categories of traits in the three study species. These results suggest that maternal oviposition behavior and thermal sensitivity of sex determination in embryos might evolve independently. Such information is critical to understand how animals with TSD will respond to rapidly changing environments that induce sex-ratio selection.

Keywords: environmental change, microevolution, nesting behavior, pivotal temperature, quantitative genetics, reptile

The sex-determining mechanism and the primary sex ratio are fundamentally intertwined. In most organisms, an individual's sex is permanently determined by its genotype at fertilization. A typical equilibrium primary sex ratio of 1:1 (a la Fisher 1930) is thus readily achieved via random meiotic segregation of the primary sex-determining element. In contrast, under environmental sex determination (ESD), an environmental factor after fertilization initiates the sex-determining cascade, which then permanently determines the individual's sex (Refsnider and Janzen 2016). In this case, the distribution of the environmental variable affects the primary sex ratio and, therefore, the efficacy of sex-ratio selection.

This latter observation motivated Bulmer and Bull (1982) to mathematically model sex-ratio dynamics under ESD. They focused on the two core categories of traits involved in temperature-dependent sex determination (TSD, the most common form of ESD) that could be subject to sex-ratio selection: (1) maternal choice of nest thermal environment and (2) sensitivity of embryonic sex determination to temperature. Bulmer and Bull (1982) developed these quantitative genetic models to explore the independent evolutionary impacts of these categories of traits. Notably, potential covariances between them were not considered.

Much empirical work since this theoretical advance has focused on quantifying phenotypic effects of single traits on offspring sex ratio in the field. Fewer studies have estimated univariate quantitative genetic parameters for maternal behavioral and embryonic physiological traits that would be most germane to addressing microevolution of TSD and TSD-like mechanisms in the context of the Bulmer and Bull (1982) models (Table 1). Moreover, with one exception (St. Juliana et al. 2004), the existence of phenotypic or genetic covariances between these two categories of traits has not been assessed formally.

Remarkable phenotypic variation exists in these important traits. Occurring in a variety of vertebrates (but primarily reptiles), TSD not surprisingly is accompanied by tremendous diversity in oviposition preferences with varying thermal characteristics, from relatively deep burrows to mounds of vegetation to sand beaches to leaf litter. Variation in this nesting behavior is mirrored in the assortment of physiological patterns of TSD (Refsnider and Janzen 2016). Some species have pattern Ia TSD (cooler incubation temperatures yield male offspring and warmer incubation temperatures produce females), others have pattern Ib TSD (the mirror image of pattern Ia), and still others have pattern II TSD (females produced at the thermal extremes and males at intermediate temperatures). Even intraspecifically, aspects of oviposition behavior and the pattern of TSD can vary within and among populations. For example, shade levels over nests of a turtle with TSD are greater in southern populations and decline as latitude increases; the converse is observed for sensitivity of embryonic sex determination to temperature across this same geographic range (Ewert et al. 2005). Even within populations, choice of nest thermal properties (e.g., Janzen and Morjan 2001) and the slope and intercept of thermally-linked sex-determining reaction norms (e.g., Rhen et al. 2011) vary considerably among individuals. The diversity in these behavioral and physiological traits implies considerable evolutionary capacity to respond to sex-ratio selection under TSD. But do these traits evolve independently or jointly to influence sex-ratio evolution?

Quantifying covariances is important to advance understanding of the microevolutionary potential of suites of traits. In particular, genetic covariances (among other possible factors; Futuyma 2010) arguably can restrain or facilitate the adaptive evolution of individual traits beyond the univariate effects of additive genetic variances alone (Lande and Arnold 1983; Agrawal and Stinchcombe 2009; but see Conner 2012). For example, genetic correlations

between key traits in several plant populations are not aligned with the direction of selection induced by climate warming, thereby retarding the rate of adaptive multivariate evolution (Etterson and Shaw 2001). Even phenotypic covariance estimates can be useful for exploring microevolutionary dynamics, as they frequently (but not universally) appear to mirror underlying genetic covariances (Cheverud 1988; Roff 1995; Kruuk et al. 2008; Dochtermann 2011; Brommer 2013).

To address the capacity for multivariate microevolution of sex ratio under TSD, we quantified the covariation of maternal nesting behavior in the field and offspring sex ratios under controlled thermal conditions in three distantly-related species of reptiles with TSD: two turtle species (*Chrysemys picta* and *Chelydra serpentina*) and one lizard (*Amphibolurus muricatus*). In these species, even small shifts in environmental temperatures dramatically alter offspring cohort sex ratio in the wild (e.g., Refsnider and Janzen 2016), thus inducing sex-ratio selection. We calculated covariances under the expectation that, to facilitate evolution in response to such sex-ratio selection, measures of maternal choice of nest thermal environment and sensitivity of embryonic sex determination to temperature should covary positively (i.e., evolution along lines of least resistance a la Schluter 1996). That is, the maternal nesting propensity to yield offspring of a given sex should correspond with the embryonic propensity to be that sex across environments. Alternatively, a negative covariance could be favored by selection to maintain a particular primary sex ratio if this sex ratio was currently in the vicinity of its optimum. Lack of such covariances presumably would imply independent evolutionary trajectories for these two categories of traits affecting primary sex ratio under TSD.

Methods

The data that we analyze here in a microevolutionary context were obtained in the course of experiments designed to address different questions and, accordingly, detailed methods can be found elsewhere (Mitchell et al. 2017; St. Juliana et al. 2004; Warner et al. 2008, respectively). Below, we provide methodological information from those experiments that is most apropos to the statistical analyses conducted for the present study and the interpretations of the results. We also survey the literature and compile published estimates of quantitative genetic parameters for traits that influence offspring sex ratio in species with thermally-sensitive sex determination. For studies with multiple estimates, we list each estimate and denote the treatment or data subset.

Painted turtle (Chrysemys picta)

Painted turtles are broadly distributed across southern Canada and from the northwestern United States through the eastern two-thirds of the country, occurring in mostly still or slow-moving freshwater habitats. This species has pattern Ia TSD; the focal study population along the backwaters of the Mississippi River in northern Illinois has a pivotal temperature of sex determination (i.e., the constant incubation temperature at which a 1:1 sex ratio is produced) of $\sim 27.7^{\circ}\text{C}$ (Refsnider et al. 2014). Females at this field site annually lay 1-3 clutches of ~ 10 eggs each from late May through early July (Schwanz et al. 2010a).

For this study (Mitchell et al. 2017), adult *C. picta* were captured from the field site and transported to the Iowa State University (ISU) Horticulture Station. In the fall of 2011, the pond was emptied and turtles brought into the laboratory to hibernate in plastic water filled tubs maintained at 4°C . Thereafter, half the turtles were released into the pond on 25 March 2012 and the other half on 13 April 2012. In May and June 2012, the adjacent terrestrial area was observed for nesting turtles and fresh nests. As a measure of nest-site choice, a hemispherical

photograph of the sky was taken for each nest to quantify percent of canopy openness, which is a proxy for nest temperatures and, correspondingly, offspring sex ratios for *C. picta* (Mitchell et al. 2013). Release date had no influence on nesting behaviors (Mitchell et al. 2017), so all 42 nests obtained from this experiment were pooled for statistical analyses in this paper.

All eggs were removed from each nest, placed in damp soil, and transported to the laboratory the same day. Eggs were assigned randomly to covered plastic shoeboxes filled with moistened vermiculite (-150 kPa), which were rehydrated weekly to maintain relatively constant hydric conditions. Every other day, shoeboxes were rotated within an incubator set to maintain a constant 28°C to minimize potential effects of any thermal gradients. After hatching, the sex of the neonates was assessed by macroscopic examination of the gonads.

Common snapping turtle (*Chelydra serpentina*)

This species is also wide ranging, inhabiting most bodies of fresh water in the eastern two-thirds of North America through Central America into northern South America. Snapping turtles have pattern II TSD; the focal study population occupies the same aquatic locality as the *C. picta* population, but has pivotal temperatures of sex determination of ~21.5°C and ~27.5 °C (O’Steen and Janzen 1999), although the lower temperature is infrequently approached in field nests (Janzen 2008) and is near the minimum for successful development. Females at this field site annually lay a single clutch of ~30-80 eggs from late May to late June (St. Juliana et al. 2004).

As described in St. Juliana et al. (2004), a sand prairie was patrolled for nesting turtles and freshly constructed nests in June 2001. Upon encountering a nest, a spherical densiometer was used to measure the proportion of overstory vegetation cover in the four cardinal directions, as this environmental factor is a proxy for nest temperatures and, correspondingly, offspring sex

ratios for *C. serpentina* (Kolbe and Janzen 2002). As a measure of nest-site choice, the proportion of total shade values (i.e., the sum of all four densiometer readings for a nest) was subtracted from 1 and multiplied by 100 to calculate a value analogous to the “percent of canopy openness” measure recorded from hemispherical photography for *C. picta* nests.

Twelve eggs were removed from each nest, 10 of which were placed in damp sand and transported to ISU within 1-4 days of oviposition. Eggs were assigned randomly to covered plastic shoeboxes filled with moistened vermiculite (-150 kPa), which were rehydrated weekly to maintain relatively constant hydric conditions. Every other day, shoeboxes were rotated within the incubator (set to maintain a constant 27.6°C) to minimize potential effects of any thermal gradients. Inadvertently, the incubator deviated to ~30°C for about 10 days, such that the average temperature during incubation was 28.37±0.18°C, which still produced both sexes. After hatching, the sex of the neonates was assessed by macroscopic examination of the gonads.

Jacky dragon (*Amphibolurus muricatus*)

This lizard inhabits coastal heathland in southeast Australia. Jacky dragons have a pattern of TSD similar to that of *C. serpentina*, but with a mix of males and females at intermediate temperatures (Harlow and Taylor 2000; Warner and Shine 2005). A balanced primary sex ratio is produced at a constant 28°C, with female biases above and below this temperature, albeit natural nests rarely average temperatures above 28°C (Warner and Shine 2008; 2011). Female jacky dragons lay 2-3 clutches of 3-9 eggs over a single nesting season, which lasts from October to February.

Adult dragons were collected near Sydney in 2003 and 2004, and housed in 2mx2m field enclosures that each included a regularly watered mound of sand (5-20cm deep) covering a

0.5m x 0.5m area (Warner et al. 2008). Each sand mound was partially covered with vegetation, which gave dragons the ability to select among a relative continuum of shaded and open microenvironments. Upon discovery of a nest, eggs were removed for laboratory incubation, a Thermocron iButton temperature logger was placed into the empty nest cavity, and the cavity was re-covered with sand. Temperature was recorded hourly during the first two-thirds of incubation, which encompasses the developmental period when sex is determined in this species (Shine et al. 2007). To measure nest-site choice, constant temperature equivalents (CTE) were first calculated for each nest site (Georges 1989), with a model parameterized for agamid lizards; the critical thermal minimum was 17.2°C (Warner and Shine 2011) and the critical thermal maximum was 36°C (Quinn et al. 2007). Nests laid earlier in the season are cooler than those laid later, so residuals were computed from a quadratic regression of CTE on oviposition date. Positive residuals represent nest sites that were relatively warm, given their oviposition date, whereas negative residuals represent relatively cool nest sites. These residual values were used as our metric for nest-site choice.

At the University of Sydney, the eggs were half buried in moistened vermiculite (-200 kPa) contained in covered glass jars. The jars were rotated within and among incubators (maintained at 28°C) three times weekly to minimize potential effects of any thermal gradients. Upon hatching, offspring sex was evaluated by eversion of hemipenes.

Statistics

To address our primary question regarding multivariate microevolutionary potential, we assessed measures of behavior and physiology linked to sex-ratio control under TSD (sensu Bulmer and Bull 1982) for each species. For maternal choice of nest thermal environment, we used the

measure of canopy openness over the nest at oviposition for each female for the two turtle species and the relative warmth of the nest cavity for each female for the lizard species. We calculated relative warmth by extracting the residuals from a quadratic regression of CTE on oviposition date (equation: $CTE = -0.00078(\text{oviposition day})^2 + 0.55(\text{oviposition day}) - 70.89$). For sensitivity of clutch sex ratio to temperature, we used the proportion of male offspring for each nest produced near the (primary) pivotal temperature in the lab. In doing so, note that this clutch-level trait can be viewed in substantial part as a property of the mother (see Discussion section for further consideration of this issue).

In all, we had 42 data points for *C. picta*, 25 data points for *C. serpentina*, and 48 data points for *A. muricatus*. To evaluate the covariance between the relevant measure of nest-site choice and clutch sex ratio near the pivotal temperature, we used a generalized linear model with a logit link function. For the two species with pattern II TSD, we also fit a 2nd-order polynomial regression to these data to assess a possible non-linear fit. We performed statistical analyses in SAS (version 9.4) and created graphics in SigmaPlot (version 13.0).

Results

The ranges of values for nest-site choice for each species and for clutch sex ratio for each species were considerable in all cases. The phenotypic variances for these traits, especially the nesting variables, were substantial (Table 2). Thus, it is unlikely that our power to detect covariance patterns was restricted by distributionally-truncated data sets. We also had substantive sample sizes for each species (Table 2).

Despite the richness of the three data sets, in no case was our measure of maternal choice of nest thermal environment related to our measure of sensitivity of embryonic sex determination to temperature at the clutch level (Table 3). Estimated covariances between these two categories of traits were essentially zero, whether we fit to the data a logistic regression or, for the two species with pattern II TSD, a 2nd-order polynomial regression (Table 3, Fig. 1). Nonetheless, eggs deposited in nests with <70% canopy openness (i.e., with more overstory vegetation cover) at oviposition for both turtle species appeared to be predisposed in the laboratory to produce males in *C. picta* (Fig. 1A) and females in *C. serpentina* (Fig. 1B); in contrast, clutches of eggs produced by females of both species that were oviposited into less shaded locations yielded a broad range of offspring sex ratios when incubated in the laboratory.

The collection of literature estimates of quantitative genetic parameters for traits that influence sex ratio in taxa with thermally-sensitive sex determination reveals the full gamut of outcomes (Table 1). Repeatability of nesting behaviors for four turtles and one lizard were generally small but often statistically significant, which is notable in that 42 of 43 estimates derived from free-ranging animals in the wild. Heritabilities for these same traits were available only for *C. picta* and were similar in magnitude to the small repeatabilities (McGaugh et al. 2010). For embryonic thermal sensitivity of sex determination, quantitative genetic estimates mainly (40 of 42) originated from laboratory studies (but see McGaugh et al. 2011) and tended to be substantive ($h^2 > 0.3$) except where paternity or expected variable thermal conditions in nature were incorporated explicitly. Several studies that did not calculate explicit quantitative genetic parameters nonetheless detected evidence for inheritance of sex-ratio tendency in all but one instance (i.e., Valenzuela and Janzen 2001). Finally, Janzen (1992) and Rhen and Lang (1998) explored genetic correlations between sex-ratio tendency of clutches at different incubation

temperatures within several species, nearly always calculating significant positive estimates. It is worth noting that the vast majority of these results reported in the literature derive from reptiles, particularly from just three species of turtles.

Discussion

Our results provide evidence that maternal nesting behavior and the thermal sensitivity of embryonic sex determination at the clutch level do not covary phenotypically within three distantly related reptile species with TSD. Assuming the concordant absence of genetic covariances as well, our findings suggest that both of these important factors for sex-ratio selection under TSD should be free to evolve independently of each other. As nesting behavior and TSD reaction norms exhibit considerable phenotypic variance and show some level of heritability in various organisms with thermally-sensitive sex determination, microevolution in response to sex-ratio selection can conceivably occur via either of these cospecialized pathways (sensu Peiman and Robinson 2017).

Bulmer and Bull (1982) concluded that, despite being expressed only in females, nesting behavior is more likely to respond evolutionarily to sex-ratio selection than is embryonic thermal sensitivity under TSD. To a considerable extent, this is because heritability of the latter traits in the wild are limited by variation in temperature among nests. For example, a nest constructed in completely unshaded (i.e., hot) or shaded (i.e., cold) habitat likely would produce a 100% single-sex sibship regardless of the genetic predispositions of the offspring. One might also expect behavior, more so than physiology, to be more responsive to selection via expressing within-generation plasticity (e.g., Schwanz and Janzen 2008). Indeed, if species with TSD can respond to sex-ratio selection via aspects of oviposition-site choice, then the opportunity for selection to

act on thermal sensitivity of embryos is mitigated (a la Huey et al. 2003; Muñoz and Losos 2018). Thus, as a null expectation, maternal behavioral changes conceivably should predominate as a phenotypic response to sex-ratio selection under TSD relative to embryonic physiological changes. The literature, sparse as it is, seems to bear out this prediction (Bull et al. 1982a; Ewert et al. 2005; Doody et al. 2006; Refsnider et al. 2014; but see Conover and Van Voorhees 1990).

The quantitative genetic literature to date affirms that the heritability of TSD-related traits in the wild—or adjusted for such—tends to be small but, often enough, significantly > 0 (Table 1). Thus, the capacity for TSD to exhibit a microevolutionary response to sex-ratio selection is frequently present but presumably minimal. Moreover, for species with pattern II TSD, substantial positive genetic correlations for offspring sex ratio at different incubation temperatures imply a possible restraint on the capacity of such patterns of TSD (i.e., overall sex-ratio reaction norms) to evolve swiftly (see also Janzen 2008). Therefore, negligible quantitative genetic variation for various traits related to TSD, along with potential constraints on the architecture of sex-ratio reaction norms, suggest that the pace of evolution especially for embryonic traits related to TSD should be slow. These findings reinforce the likely selective value of plasticity in oviposition behavior in taxa with TSD (see above) that experience disrupted thermal environments or other factors that alter primary sex ratio. Nonetheless, the quantitative genetic literature on TSD-related traits is minimal and taxonomically biased, so more work on this topic on a broader, targeted set of taxa is warranted to best support generalizations.

Our findings probably yield few insights into molecular mechanisms underpinning traits linked to TSD. Still, the lack of phenotypic covariances between maternal and clutch-level embryonic TSD traits in our three targeted species suggests independent gene networks or modules involved. Perhaps this conclusion is unsurprising given the maternal physiology likely

involved with “thermal” sensing of a nest site relative to that involved with embryos transducing a thermal cue to regulate a sex-determining developmental program. Regardless, our understanding of the molecular basis of TSD is growing, as recent meticulous studies have identified genes involved in sex-determining pathways in several reptiles with TSD (e.g., Schroeder et al. 2016; Deveson et al. 2017; Ge et al. 2018). It is unclear if research on the molecular basis of thermally-based oviposition-site choice in reptiles with TSD will be similarly successful. However, as reptiles with TSD exhibit repeatable nesting behavior (Table 1) and can learn to navigate changing ultraviolet landscapes (e.g., Roth and Krochmal 2015), creative work on this topic using a thermal gradient (e.g., Bull et al. 1988) might provide an exemplar approach to begin dissecting such behavioral traits at the molecular level.

It is important to acknowledge that our studies—the first of their kind in taxa with TSD—have weaknesses that limit evolutionary inferences. We have placed our analyses in a quantitative genetic framework, yet we do not have information on the genealogical relationships among the adult females within each study to explicitly estimate genetic (co)variances. Although phenotypic covariances often can reflect genetic covariances (Cheverud 1988; Roff 1995; Kruuk et al. 2008; Dochtermann 2011; Brommer 2013), and therefore can provide insights into microevolutionary dynamics, perforce they are not the same thing. Even so, we estimated covariances at the maternal/clutch level, not the individual level, which renders them closer to being genetic covariances. Furthermore, the frequency of within-clutch multiple paternity in our three species is substantive (>30% in all cases; Pearse et al. 2002; Galbraith et al. 1993; Warner et al. 2010), thereby enhancing the likelihood that phenotypic and genetic estimates are similar. Moreover, the extensive phenotypic variances we detected are consistent with negligible constraint on direct sex-ratio evolution.

Other issues regarding the merit of our synthetic work include questions about the relevance of the phenotypes we measured to the maternal and embryonic traits that actually contribute to sex-ratio evolution under TSD in these species in the wild. For example, is our gauge of sensitivity of embryonic sex determination to temperature (i.e., the intercept of the sex-ratio reaction norm)—quantified under constant incubation conditions—an adequate or appropriate measure? Should such sensitivity for each clutch instead be characterized by different traits such as the range of constant temperatures over which both sexes are produced (the slope of the sex-ratio reaction norm; e.g., Hulin et al. 2009), by the full sex-ratio reaction norm (e.g., Murren et al. 2014; Pezaro et al. 2017), or by fluctuating temperatures that reflect natural thermal environments in nests (e.g., Paitz et al. 2010)? Even the cross-generational transmission of sensitivity of embryonic sex determination to temperature can be subject to potential epigenetic effects that alter traditional interpretations of the inheritance of these traits (e.g., Warner et al. 2013). Regardless, positive genetic correlations between clutch sex ratio measured at adjacent incubation temperatures in *C. picta* and *C. serpentina* (Table 1) imply robustness for its use as a proxy measure of sensitivity of embryonic sex determination to temperature. In addition, the among-clutch variation in sex ratios at pivotal temperatures (ranging from 0 – 100% male for *C. picta* and *A. muricatus*; Fig. 1) further illustrates that our measure of the thermal sensitivity of sex determination for each clutch is appropriate.

Several other potential concerns are evident. As with sensitivity of embryonic sex determination to temperature, the manner in which we characterized maternal choice of nest thermal environment may be incomplete or inadequate. Beyond shade cover, the date on which a nest was laid (e.g., Ewert et al. 2005), the moisture environment of a nest (e.g., Lolavar and Wyneken 2017), and the depth, slope, aspect, and albedo of a nest (e.g., Hays et al. 2001) all can

influence the nest thermal environment and, hence, offspring sex ratio. The maternal effects of among-female differential allocation of yolk hormones or epigenetic factors (e.g., Bowden et al. 2000) also could affect nest sex ratio and therefore be subject to sex-ratio selection. Even the experimental set-up to assess nesting behavior may be questionable for *C. picta* and *A. muricatus*, because we used semi-natural enclosures in both cases. In the former instance, we cannot know if the nesting turtles perceived the artificial shade cover objects in the same way as natural vegetation cover when identifying nesting sites. Similarly, for the lizards, variation detected in their nest-site choice could be attributable to random nesting and not to active decision-making. Despite these potential issues, shade cover should provide females with a relatively reliable cue of the near-term thermal environment of a nest site. Moreover, females of many reptiles with TSD return repeatedly to similar nest environments (Janzen and Morjan 2001; Freedberg et al. 2005; Refsnider et al. 2010; Jensen et al. 2018), suggesting the potential for covariances to arise between such traits and others conceivably involved in sex-ratio selection.

Apart from any weaknesses with our work, the core question addressed in this paper is more than academic. The majority of species with TSD are imperiled, hence ideal conservation and management efforts require the most informed understanding of their evolutionary potential in the context of TSD as overall climate and local habitats are rapidly being altered (Mitchell and Janzen 2010; Refsnider and Janzen 2016). Indeed, a growing body of research is documenting the substantial sensitivity to climatic thermal variation of offspring sex ratios in wild populations of diverse species with TSD (Janzen 1994; Mitchell et al. 2008; Telemeco et al. 2009, Pen et al. 2010; Schwanz et al. 2010b; Jensen et al. 2018) and the downstream effects on adult population structure (Schwanz et al. 2010a; Grayson et al. 2014; Holleley et al. 2015). Our results suggest that thermally-linked maternal oviposition behavior and thermal sensitivity of sex determination

in embryos might evolve independently. Nonetheless, low estimates of quantitative genetic parameters for these traits in species with thermally-sensitive sex determination imply that population responses to environmentally-induced sex-ratio selection typically may be accomplished more readily and swiftly via phenotypic plasticity. Accordingly, conservation and management efforts should actively provide an array of thermal environments to allow the natural expression of variation in nest-site choice and subsequent embryonic sexual differentiation in species with TSD.

Funding

This work was supported by funding sources acknowledged in the original papers. Our current collaborative work on these questions is supported by National Science Foundation grant IOS-1257857.

Acknowledgements

We are grateful to Anne Bronikowski for the opportunity to participate in the AGA symposium and to contribute this paper; to various entities for permission to conduct the research (permits for the studies are acknowledged in the original papers); and to the Janzen Lab and three anonymous reviewers for constructive comments on drafts of the manuscript.

Data Availability

We deposited the raw data underlying our analyses in Table 2, Table 3, and Figure 1 in Dryad.

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Table 1: Estimated quantitative genetic parameters for maternal behavioral traits and embryonic physiological traits potentially related to sex-ratio selection in animals with thermally-sensitive sex determination. R =repeatability, h^2 =heritability, r_G =genetic correlation. Traits in bold were measured under field conditions, whereas parameters in bold represent reports of $P < 0.05$. Consult original references for details of the studies and statistical analyses. For studies containing multiple estimates, we listed each estimate and denoted the treatment or data subset.

| Taxon/species | Trait | Parameter, SE or CI | Notes | Reference |
|-------------------------------|--------------|---------------------|-----------------------------|------------------------------------|
| Copepod | Sex tendency | $h^2 = 0.12, 0.39$ | 15°C (summer) | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | | | | |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0, 0.17$ | 22°C (summer) | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.24, 0.058$ | 15°C (fall) | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.16, 0.045$ | 22°C (fall) | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.13, 0.04$ | Pooled | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.10, 0.033$ | 15°C (summer) corrected | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0, 0.014$ | 22°C (summer) corrected | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.19, 0.05$ | 15°C (fall) corrected | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.12, 0.039$ | 22°C (fall) corrected | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.10, 0.034$ | Pooled corrected | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.31, 0.216$ | | Voordouw and Anholt 2002 |
| <i>Tigriopus californicus</i> | Sex tendency | $h^2 = 0.36, 0.225$ | | Voordouw and Anholt 2002 |
| Fish | | | | |
| <i>Dicentrarchus labrax</i> | Sex tendency | $h^2 = 0.62, 0.12$ | | Vandeputte et al. 2007 |
| <i>Dicentrarchus labrax</i> | Sex tendency | $h^2 = 0.52, 0.13$ | | Vandeputte et al. 2007 |
| <i>Dicentrarchus labrax</i> | Sex tendency | $h^2 = 0.72, 0.20$ | | Vandeputte et al. 2007 |
| <i>Oreochromis niloticus</i> | Sex ratio | $h^2 = 0.63$ | High male sex ratio line | Wessels and Hoerstgen-Schwark 2011 |
| <i>Oreochromis niloticus</i> | Sex ratio | $h^2 = 0.84$ | Low male sex ratio line | Wessels and Hoerstgen-Schwark 2011 |
| <i>Oreochromis niloticus</i> | Sex ratio | $h^2 = 0.73$ | Divergence of the two lines | Wessels and Hoerstgen-Schwark 2011 |
| <i>Menidia menidia</i> | Sex ratio | NA | Paternal effect | Conover and Heins 1987 |
| <i>Menidia menidia</i> | Sex ratio | NA | Paternal effect | Conover and Heins 1987 |
| <i>Menidia menidia</i> | Sex ratio | NA | Paternal effect | Conover and Heins 1987 |
| <i>Menidia menidia</i> | Sex ratio | NA | Population effect | Conover et al. 1992 |
| Crocodylian | | | | |

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|-----------------------------------|-----------------------|-------------------------|-----------------------------|-------------------------|
| <i>Alligator mississippiensis</i> | Sex ratio | NA | Family effect | Rhen and Lang 1998 |
| <i>Alligator mississippiensis</i> | Sex ratio | NA | Temperature effect | Rhen and Lang 1998 |
| <i>Alligator mississippiensis</i> | Sex ratio | NA | Family x temperature effect | Rhen and Lang 1998 |
| <i>Alligator mississippiensis</i> | Sex ratio | $r_G = -0.046$ | 31.8°C x 33.8°C | Rhen and Lang 1998 |
| <i>Alligator mississippiensis</i> | Sex ratio | $r_G = 0.128$ | 31.8°C x 34.3°C | Rhen and Lang 1998 |
| <i>Alligator mississippiensis</i> | Sex ratio | $r_G = 0.81$ | 33.8°C x 34.3°C | Rhen and Lang 1998 |
| Lizard | | | | |
| <i>Eublepharis macularius</i> | Nest temperature | R = 0.2 | | Bull et al. 1988 |
| <i>Eublepharis macularius</i> | Sex tendency | $h^2 = 0.37, 0.33$ | 30°C | Rhen et al. 2011 |
| <i>Eublepharis macularius</i> | Sex tendency | $h^2 = 0.09, 0.19$ | 32.5°C | Rhen et al. 2011 |
| <i>Eublepharis macularius</i> | Sex tendency | $h^2 = 0.40, 0.27$ | 30°C | Rhen et al. 2011 |
| <i>Eublepharis macularius</i> | Sex tendency | $h^2 = 0, 0.22$ | 32.5°C | Rhen et al. 2011 |
| <i>Eublepharis macularius</i> | Sex tendency | $h^2 = 0.257, 0.115$ | 30°C | Rhen et al. 2011 |
| <i>Eublepharis macularius</i> | Sex tendency | $h^2 = 0.061, 0.041$ | 32.5°C | Rhen et al. 2011 |
| <i>Eublepharis macularius</i> | Nest temperature | NA | | Bragg et al. 2000 |
| <i>Hemiteconyx caudicinctus</i> | Nest temperature | NA | | Bragg et al. 2000 |
| Turtle | | | | |
| <i>Graptemys ouachitensis</i> | Sex ratio | $h^2 = 0.82, 0.31-1$ | | Bull et al. 1982b |
| <i>Graptemys ouachitensis</i> | Sex ratio | $h^2 = 0.315$ | | McGaugh and Janzen 2011 |
| <i>Chelydra serpentina</i> | Sex ratio | $h^2 = 0.60, 0.1008-1$ | 27.5°C | Janzen 1992 |
| <i>Chelydra serpentina</i> | Sex ratio | $h^2 = 0.76, 0.3009-1$ | 28.0°C | Janzen 1992 |
| <i>Chelydra serpentina</i> | Sex ratio | $h^2 = 0.34, 0-1$ | 28.5°C | Janzen 1992 |
| <i>Chelydra serpentina</i> | Sex ratio | $h^2 = 0.56, 0.2569-1$ | Pooled | Janzen 1992 |
| <i>Chelydra serpentina</i> | Sex ratio | $r_G = 0.73, 0.47-0.99$ | 27.5° C x 28.0° C | Janzen 1992 |
| <i>Chelydra serpentina</i> | Sex ratio | $r_G = 0.52, 0.07-0.96$ | 27.5° C x 28.5° C | Janzen 1992 |
| <i>Chelydra serpentina</i> | Sex ratio | $r_G = 0.67, 0.37-0.97$ | 28.0° C x 28.5° C | Janzen 1992 |
| <i>Chelydra serpentina</i> | Sex ratio | $h^2 = 0.193$ | | McGaugh and Janzen 2011 |
| <i>Chelydra serpentina</i> | Sex ratio | NA | Family effect | Rhen and Lang 1998 |
| <i>Chelydra serpentina</i> | Sex ratio | NA | Temperature effect | Rhen and Lang 1998 |
| <i>Chelydra serpentina</i> | Sex ratio | NA | Family x temperature effect | Rhen and Lang 1998 |
| <i>Chelydra serpentina</i> | Sex ratio | $r_G = 0.22$ | 27.5° C x 28.0° C | Rhen and Lang 1998 |
| <i>Chelydra serpentina</i> | Sex ratio | $r_G = -0.19$ | 27.5° C x 28.5° C | Rhen and Lang 1998 |
| <i>Chelydra serpentina</i> | Sex ratio | $r_G = 0.62$ | 28.0° C x 28.5° C | Rhen and Lang 1998 |
| <i>Chelydra serpentina</i> | Sex ratio | NA | Female effect | St. Juliana et al. 2004 |
| <i>Chrysemys picta</i> | Nest vegetation cover | R = 0.18 | All females | Janzen and Morjan 2001 |

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|------------------------|------------------------------|--|-----------------------------|----------------------------|
| <i>Chrysemys picta</i> | Nest vegetation cover | R = 0.21 | Females with ≥ 3 nests | Janzen and Morjan 2001 |
| <i>Chrysemys picta</i> | Sex ratio | NA | | Valenzuela and Janzen 2001 |
| <i>Chrysemys picta</i> | Nest location | R = 0.03 | Mantel | Valenzuela and Janzen 2001 |
| <i>Chrysemys picta</i> | Nest vegetation cover | R = 0.01 | Mantel | Valenzuela and Janzen 2001 |
| <i>Chrysemys picta</i> | Nesting date | R = 0.03 | | Schwanz and Janzen 2008 |
| <i>Chrysemys picta</i> | Nesting date | R = 0.022, -0.083-0.124 | Cold winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nesting date | R = 0.057, -0.083-0.197 | Mild winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nesting date | R = 0.118, 0.014-0.222 | Hot winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nesting date | R = 0.060, 0.026-0.095 | Pooled | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nesting date | $h^2 = 0.060, -0.131-0.250$ | Cold winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nesting date | $h^2 = 0.103, -0.073-0.278$ | Mild winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nesting date | $h^2 = 0.166, 0.020-0.313$ | Hot winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nesting date | $h^2 = 0$ | Pooled | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | R = 0.260, 0.146-0.374 | Cold winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | R = 0.072, -0.021-0.166 | Mild winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | R = 0.181, 0.097-0.266 | Hot winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | R = 0.140, 0.097-0.182 | Pooled | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | NA | Cold winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | NA | Mild winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | $h^2 = 0.188, 0.104-0.271$ | Hot winters | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | $h^2 = 0.043, -0.126-0.211$ | Pooled | McGaugh et al. 2010 |
| <i>Chrysemys picta</i> | Nest vegetation cover | $h^2 = 0.057$ | | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.460, 0.043-1$ | Constant temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.517, 0.074-1$ | Fluctuating temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.446, 0.024-0.799$ | Constant temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.576, 0.228-1$ | Fluctuating temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.171$ | Constant temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.221$ | Fluctuating temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.186$ | Constant temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.240$ | Fluctuating temperature | McGaugh and Janzen 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.351, 0.164-0.832$ | | McGaugh et al. 2011 |
| <i>Chrysemys picta</i> | Sex tendency | $h^2 = 0.173, 0-0.628$ | | McGaugh et al. 2011 |
| <i>Chrysemys picta</i> | Sex ratio | NA | Family effect | Rhen and Lang 1998 |
| <i>Chrysemys picta</i> | Sex ratio | NA | Temperature effect | Rhen and Lang 1998 |
| <i>Chrysemys picta</i> | Sex ratio | NA | Family x temperature effect | Rhen and Lang 1998 |

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|-------------------------------|---|----------------------|--|------------------------------|
| <i>Chrysemys picta</i> | Sex ratio | $r_G = 0.42$ | 28.5° C x 29.0° C | Rhen and Lang 1998 |
| <i>Dermochelys coriacea</i> | Nest distance to highest tide line | R = 0.18 | Females with ≥ 3 nests | Kamel and Mrosovsky 2004 |
| <i>Dermochelys coriacea</i> | Nest distance to highest tide line | R = 0.21 | All females | Kamel and Mrosovsky 2004 |
| <i>Dermochelys coriacea</i> | Nest distance to water line | R = 0.20 | | Kamel and Mrosovsky 2004 |
| <i>Dermochelys coriacea</i> | Nest distance to water line | R = 0.18 | | Kamel and Mrosovsky 2004 |
| <i>Eretmochelys imbricata</i> | Nest distance to water line | R = 0.23 | | Kamel and Mrosovsky 2005 |
| <i>Eretmochelys imbricata</i> | Nest distance to forest | R = 0.40 | | Kamel and Mrosovsky 2005 |
| <i>Eretmochelys imbricata</i> | Nest location | R = 0.62 | | Kamel and Mrosovsky 2005 |
| <i>Eretmochelys imbricata</i> | Nest vegetation cover | R = 0.71 | | Kamel and Mrosovsky 2005 |
| <i>Eretmochelys imbricata</i> | Nest distance to water line | R = 0.35 | Within year | Kamel and Mrosovsky 2006 |
| <i>Eretmochelys imbricata</i> | Nest distance to forest | R = 0.36 | Within year | Kamel and Mrosovsky 2006 |
| <i>Eretmochelys imbricata</i> | Nest location | R = 0.49 | Within year | Kamel and Mrosovsky 2006 |
| <i>Eretmochelys imbricata</i> | Nest vegetation cover | R = 0.69 | Within year | Kamel and Mrosovsky 2006 |
| <i>Eretmochelys imbricata</i> | Nest distance to vegetation | R = 0.58 | Within year | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to highest tide line | R = 0.52 | Within year | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to water line | R = 0.54 | Within year | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest location | R = 0.65 | Within year | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to vegetation | R = 0.55 | Within and between years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to highest tide line | R = 0.52 | Within and between years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to water line | R = 0.54 | Within and between years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest location | R = 0.68 | Within and between years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to vegetation | R = 0.65 | Excludes sand slope nests within years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to highest tide line | R = 0.58 | Excludes sand slope nests within years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to water line | R = 0.55 | Excludes sand slope nests within years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest location | R = 0.69 | Excludes sand slope nests within years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to vegetation | R = 0.61 | Excludes sand slope nests within and between years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to highest tide line | R = 0.57 | Excludes sand slope nests within and between years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest distance to water line | R = 0.56 | Excludes sand slope nests within and between years | Santos et al. 2016 |
| <i>Eretmochelys imbricata</i> | Nest location | R = 0.81 | Excludes sand slope nests within and between years | Santos et al. 2016 |
| <i>Caretta caretta</i> | Nesting date | R = 0.03 | | Reneker and Kamel 2016 |
| <i>Podocnemis lewyana</i> | Sex tendency | $h^2 = 0.75, 0.15-1$ | | Gallego-Garcia and Paez 2016 |

Table 2: Basic statistics for observed nesting behaviors in the field (*Chelydra serpentina*) and outdoor enclosures (*Chrysemys picta* and *Amphibolurus muricatus*) (CTE=constant temperature equivalent) and for observed sex ratios from controlled laboratory incubation of eggs (see Methods section for details of variables).

| | N | mean | variance | minimum | maximum |
|-------------------------------|----|-------|----------|---------|---------|
| <i>Chrysemys picta</i> | | | | | |
| percent canopy open | 42 | 77 | 194 | 46 | 96 |
| proportion male | 42 | 0.70 | 0.08 | 0 | 1 |
| <i>Chelydra serpentina</i> | | | | | |
| percent canopy open | 25 | 79 | 457 | 25 | 100 |
| proportion male | 25 | 0.20 | 0.05 | 0 | 0.71 |
| <i>Amphibolurus muricatus</i> | | | | | |
| CTE (°C) | 48 | 25.72 | 2.35 | 22.6 | 28.6 |
| proportion male | 48 | 0.39 | 0.08 | 0 | 1 |
| residuals | 48 | 0 | 1.51 | -2.2 | 2.7 |

Table 3: Parameter estimates of the relationship between nesting behavior and offspring sex ratio data for three species of reptiles with temperature-dependent sex determination (TSD), derived from a generalized linear model. For *Chrysemys picta* and *Chelydra serpentina*, the slope is in units of percent canopy openness over a nest. For *Amphibolurus muricatus*, the slope is in units of residuals from the regression of the constant temperature equivalent on the oviposition date of a nest. For both *C. serpentina* and *A. muricatus*, which have pattern II TSD, we also ran quadratic models.

| | Linear $\beta \pm$ SE | P | Quadratic $\beta \pm$ SE | P |
|-------------------------------|-----------------------|------|--------------------------|------|
| <i>Chrysemys picta</i> | -0.006 \pm 0.004 | 0.10 | - | - |
| <i>Chelydra serpentina</i> | 0.007 \pm 0.014 | 0.63 | - | - |
| | 0.000 \pm 0.000 | 0.71 | -0.023 \pm 0.078 | 0.77 |
| <i>Amphibolurus muricatus</i> | -0.001 \pm 0.082 | 0.98 | - | - |
| | 0.001 \pm 0.088 | 0.98 | -0.029 \pm 0.067 | 0.66 |

Figure legends

Fig. 1. Relationships between thermal aspects of nests in the field and offspring sex ratio under controlled temperatures for three distantly-related reptile species with temperature-dependent sex determination. The nest thermal trait for the two turtle species (A: *Chrysemys picta* and B: *Chelydra serpentina*) was canopy cover at oviposition and for the lizard species (C: *Amphibolurus muricatus*) was the residuals of the quadratic regression of the constant temperature equivalent on oviposition date (i.e., higher residuals represent warmer nests for a given oviposition date). Offspring sex ratio data are untransformed for visualization purposes.

