A simple explanation for the evolution and maintenance of temperature-dependent sex determination

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May 5, 2020

Abstract

The Charnov-Bull model stipulates that environmental sex determination (ESD) is favoured when fitness of the sexes responds differently to the environment. However, Charnov-Bull has not yet been broadly successful in explaining the adaptive significance of temperature-dependent sex determination (TSD), a form of ESD. Specifically, there is no broad and convincing explanation for the occurrence of a sex-by-incubation temperature interaction for fitness. Here, I point out that the Trivers-Willard model, which predates Charnov-Bull, has never been applied broadly to explain the adaptive significance of TSD. Under Trivers-Willard, a sex-by-environment interaction for fitness occurs along the axis of individual condition (general health, vigour, etc), as male fitness is relatively sensitive to condition by virtue of relatively strong sexual selection; this occurs regardless of the direction of sexual-size dimorphism. I propose that incubation temperature affects condition, and TSD evolves so that males arise from relatively high-quality incubation environments; I call this the Mighty Males hypothesis. Re-examination of existing literature provides significant support to Mighty Males and its assumptions. Mighty Males can also explain why aspects of the environment other than temperature affect sex in some TSD species. Ultimately, I provide a simple explanation for TSD, and I suggest directions for future work.

Introduction

Environmental sex determination (ESD) occurs in a phylogenetically diverse group of organisms, including plants, invertebrates, fish, and reptiles. Unlike genetic sex determination (GSD), where sex is determined by genetic factors at conception, sex under ESD is determined by an environmental cue experienced by the embryo after conception. It is perhaps easy to envision how frequency-dependent selection favours GSD when each sex contributes half their genes to the next generation, but the evolution of maintenance of ESD is puzzling given the possibility of extreme sex ratio bias that would favour the rarer sex (Bull 1980). The most promising and general explanation for ESD is known as the Charnov-Bull model, where ESD may be adaptive when there is a sex by environment interaction for fitness (Charnov & Bull 1977). In other words, male fitness and female fitness must respond differently to the environment, and ESD may evolve if a reliable cue can predict the environment during the embryonic period. Consistent with the Charnov-Bull model, photoperiod affects sex in the amphipod *Gammarus duebeni* , because males benefit from increased size more than females, and hence males are born early in the season and grow to a bigger size (McCabe & Dunn 1997). In a parasitic wasp (*Lariophagus sp)* , eggs laid in large insect hosts become female, not male, as female wasps benefit more than males from increased size (fecundity) at adulthood (Charnov *et al.* 1981). Clearly, different environmental stimuli and various adaptive mechanisms can be consistent with the Charnov-Bull model. For specific types of ESD, then, the current challenge is to apply the Charnov-Bull model in a way that can generally explain the evolution and maintenance of ESD where it is taxonomically widespread.

The most common form of ESD is temperature-dependent sex determination, or TSD, which is found mainly in reptiles, but also in several fishes (Ewert *et al.* 1994; Janzen & Phillips 2006). In reptiles with TSD, the

incubation temperatures experienced by the embryo during the thermosensitive period influences sex, where the thermosensitive period comprises specific anatomical stages that occur roughly during the middle third of embryonic development (Yntema 1968, 1979; see also Girondot *et al.* 2018). Under constant temperature, the temperature-sex reaction norm is known to take three forms. The FMF pattern occurs when males are produced at intermediate temperatures and females are produced at extreme temperatures (Fig 1a). The MF pattern occurs when males are produced at cool temperatures and females at hot temperatures (Fig 1b). Finally, the FM pattern occurs when females are produced at cool temperatures and males at hot temperatures (Fig. 1c). The FMF pattern is hypothesized to be ancestral, as it subsumes the FM and MF patterns.

Because TSD is phylogenetically widespread in reptiles, an adaptive explanation that applies to most or all afflicted species has been sought (reviewed in Janzen and Paukstis 1991; Shine 1999; Valenzuela 2004; Janzen and Phillips 2006), with the general presumption that any unifying explanation would be rooted in the Charnov-Bull model (Shine 1999). Despite considerable effort (Ferguson & Joanen 1983; Ewert *et al.* 1994; Janzen 1995; Morjan & Janzen 2003; Warner & Shine 2005; Spencer & Janzen 2014), there is no adaptive explanation for TSD that is both broadly convincing and supported (Shine 1999; Janzen & Krenz 2004; Schwanz *et al.* 2016). The root of the problem is likely related, in part, to the fact that temperature is not only a cue that affects sexual differentiation, but also has profound developmental and physiological effects that encompass a myriad of other traits expressed across an individual's lifetime (Noble *et al.* 2018b). Uncovering the adaptive significance of TSD is therefore not as simple as in other ESD cases, such as photoperiodic cues, as temperature exhibits direct effects on many traits, including fitness.

Because an adaptive and broadly-applicable explanation for TSD has yet to be uncovered, the current paradigm is, arguably, that TSD persists in many reptiles because it is selectively neutral, and it is difficult to evolve GSD (Bull 1980; Girondot & Pieau 1999; Janzen & Krenz 2004). This hypothesis seems at odds with the recent evolution of GSD in several turtle lineages (Ewert & Nelson 1991), and with theory suggesting that transitions between GSD and TSD require little genetic innovation (Quinn *et al.* 2011). The hypothesis is also at odds with common and extreme biases in population sex ratios that would advantage the rarer sex (e.g., Ferguson and Joanen 1983; Schwarzkopf and Brooks 1985; Jensen et al. 2018), as well as rapid post-Wisconsinian evolution of TSD patterns with respect to climate (Ewert *et al.* 2004, 2005; but see Carter *et al.* 2019a). In the present study, I point out that a simple and surprisingly well-supported explanation for a sex-by-environment interaction under TSD has been overlooked, one that predates the Charnov-Bull model. Ultimately, I integrate classical theories in evolutionary ecology to develop a broadly applicable framework that explains the evolution and maintenance of TSD. While I focus on patterns in reptiles, the explanation may also apply to some or all TSD fishes.

Trivers-Willard and the Mighty Males Hypothesis

Trivers and Willard first proposed that sex allocation should be sensitive to the environment (Trivers & Willard 1973). In their verbal model, females produce more sons when sons are expected to become high-condition adults, where condition refers to general health, energy reserves, vigor, and overall quality. The logic is that male fitness is limited by mating opportunity, whereas female fitness is limited by gamete production (Bateman 1948). As a result, males experience stronger sexual selection (Trivers 1972; Singh & Punzalan 2018), and male reproductive success (number of successful inseminations) will be exponentially distributed with a modal value of zero (Jones *et al.* 2002) (Fig. 2a). In other words, a small proportion of males capture a large portion of the mating opportunities. Further, male phenotype is non-random with respect to the distribution of mating success, as high-condition males secure most matings (Rowe & Houle 1996). For females, however, the narrative is different. Given that female fitness is gamete limited, not mate limited, the distribution of female reproductive success (fecundity) will tend towards normality (Fig. 2b). In other words, an overwhelming majority of females will reproduce, and variation in female condition will predict variation in fecundity, as opposed to variation in reproductive failure in the case of males. In sum, male fitness is more sensitive to variation in condition than female fitness (Fig. 3a).

It follows that a sex by environment interaction for fitness will always occur if the environment predicts

condition. Most importantly, the interaction occurs in the simplest possible case, which is when the environment affects the condition of males and females in exactly the same way. The interaction occurs because males in high condition can generally expect to have greater fitness than females in the same condition, but females in low condition can generally expect to have greater fitness than males in the same condition. A parent should therefore adjust the sex of offspring if offspring condition is predictable: if high condition is predicted, then sex should be adjusted towards males, as a high-condition male is likely to provide greater fitness return than a high-condition female (Trivers & Willard 1973).

Trivers and Willard (1973) originally envisioned a polygamous mammalian system, perhaps because male-male competition for mates is obvious and intense in these species. Yet, these classical principles have since been extended to a variety of other taxa (West 2009). Indeed, an exponential distribution of male mating success – a key assumption of Trivers-Willard – is not restricted to mammals with extreme male–male combat or species with male-biased size dimorphism. The pattern is general (Arnqvist & Rowe 2005), and it is found in territorial reptiles (Trillmich 1983) as well as in other ectothermic vertebrates with no obvious signs of territorial behaviour or extreme male combat (Jones *et al.* 2002). I argue that these classic principles of sexual selection and condition-dependence also have an inexorable bearing on the evolution and maintenance of TSD. Specifically, if incubation temperature has, on average, an effect on the condition of individuals at adulthood, then the key requirement of the Charnov-Bull model, a sex by incubation temperature interaction for fitness, must occur when males are produced under favourable incubation temperatures and females under unfavourable incubation temperatures (Fig. 3b,c). The main difference between the hypothesis I propose and the Trivers-Willard hypothesis is that condition is predicted by temperature, instead of some aspect of the parental environment or phenotype (Fig. 3). This important difference also happens to be a strength of the hypothesis I propose, as it allows circumvention of a persistent criticism of Trivers-Willard: a well-understood proximate mechanism that can trigger conditional allocation to sex (Cameron *et al.* 2008). To differentiate my hypothesis from classic Trivers and Willard (1973), I refer to my hypothesis simply as the 'Mighty Males' hypothesis.

Trivers-Willard is the first general model of conditional sex allocation, so it is not surprising that Trivers-Willard features prominently in discussion of ESD (e.g., West 2009). Indeed, a few explanations for TSD in reptiles have made passing reference to, or are partly rooted in, the Trivers-Willard hypothesis (Head *et al.* 1987; Deeming *et al.* 1988; Deeming & Ferguson 1989; Ewert *et al.* 1994; Roosenburg 1996). West (2009, p. 246) even goes so far as to state "One way this [sex-by-environment interaction] can occur is if one sex gains more than the other from developing in a "better" environment, as in the classic Trivers and Willard hypothesis", but this statement is followed only by a brief discussion of studies that are limited to crocodilians, in which males guard harems as in classic Trivers-Willard (Deeming *et al.* 1988; Deeming & Ferguson 1989). What is perhaps surprising is that no study to my knowledge has explored the possibility that Mighty Males may provide a general explanation for TSD. I suggest the reason is because Trivers-Willard predates the Charnov-Bull model of ESD, it is not immediately obvious that the non-specific form of sex-by-environment interaction for fitness described by Charnov and Bull can be reconciled with sex-specific condition dependence for fitness, described by Trivers and Willard.

Assumptions of Mighty Males

A main assumption of Mighty Males is that temperature affects condition of adults. Empirical tests performed under the lens of Mighty Males are required to assess this assumption with certainty (Mitchell *et al.* 2018). However, it is well-known that incubation temperature affects a myriad of traits, including embryonic survival (Schwarzkopf & Brooks 1985; Schwanz *et al.* 2010), embryonic development rate (Georges *et al.* 2005), size at hatching (Ferguson & Joanen 1983; Janzen & Morjan 2002), juvenile growth rate (O'Steen 1998; Janzen & Morjan 2002), juvenile behaviour (Janzen 1995; Booth *et al.* 2004), juvenile immune function (Dang *et al.* 2015), post-hatching survival (Dayananda *et al.* 2017), and gamete size, reproductive physiology and behaviour of adults (Gutzke & Crews 1988; Jonsson *et al.* 2014). In fact, many other adaptive explanations for TSD in reptiles (at least those based on the Charnov-Bull model) also require a persistent effect of incubation temperature on phenotype (Shine 1999). A persistent effect on condition would not

be surprising, as a high-quality developmental environment would result in a high-quality phenotype that, for one, enjoys any physiological advantage provided by favourable incubation conditions, and also enjoys successive non-independent events that increase the individual's relative health and vigor over its lifetime (Madsen & Shine 2000).

A key requirement of Mighty Males is that variation in condition exists. Any factor that increases the variance in male condition or quality will therefore favour this mechanism of TSD. For instance, rapid maturation of males and non-overlapping generations is less compatible with Mighty Males, as rapid maturation of an evenly-aged cohort results in a low variance in male quality, even assuming all males are produced in a "good" environment. Factors that increase variance in male quality in a population includes environmental stochasticity over a protracted juvenile period, late age at maturity, long lifespan and/or a protracted period of indeterminate growth, low egg-to adult survival, and overlapping generations. Interestingly, many of the aforementioned characteristics have long been known to be associated with the evolution of TSD (Bull & Bulmer 1989; Sabath *et al.* 2016). Classically, the association between TSD and slow life histories arises because longevity and overlapping generations reduce the influence of climate on population sex ratios (Bull & Bulmer 1989); these life-history characteristics will also favour the evolution of TSD (or at least negate selection against TSD) under Mighty Males.

Finally, Mighty Males assumes that incubation temperature, not sex, will influence fitness and fitness-related traits, as the optimum incubation temperature is the same for both sexes (Figure 3b,c). Support for this assumption can therefore be sought by examining studies that use hormonal manipulations to decouple sex and incubation temperature in TSD species. There are few such studies, but those that exist generally support the assumption. In the well-studied snapping turtle *Chelydra serpentina* , an FMF species, it is well known that male-producing temperatures promote early juvenile growth (Brooks *et al.* 1991; Bobyn & Brooks 1994b, a), and hormonal manipulation reveals that it is incubation temperature, not sex, that promotes early growth (Rhen & Lang 1994, 1999). In the lizard *Amphibolurus muricatus* , another FMF species, hormonal manipulation revealed that incubation temperature influenced phenotype independent of sex, and the treatment producing the most males never performed worse than another treatment for any phenotype measured (Warner & Shine 2005). Thus, both these studies (and a few others described in subsequent sections) suggest that temperature indeed influences phenotype independent of sex.

The MF and FMF patterns

It is easy to envision how the FMF pattern aligns with Mighty Males (Fig. 3a). Recall that the FMF pattern is broadly found in turtles and crocodilians, as well as in some lizards, and is likely the ancestral condition (Fig. 1a). Under the FMF pattern, females are produced in hot and cold incubation environments, and males at intermediate temperatures. Assuming the physiology of the embryo evolves to match the average environment (e.g., Ewert 1985), then one would expect that an intermediate temperature would represent optimal incubation conditions, imparting a high-quality phenotype and high individual condition. Temperatures that deviate from the average environment, however, would produce phenotypes of a quality that is inversely proportional to the degree of deviation (e.g., Noble et al. 2018).

The framing of the MF pattern with respect to the focal hypothesis depends on the evolutionary origin of the MF pattern. In the simplest and perhaps most probable case, the FMF pattern and FM pattern are two sides of the same coin, and it suffices to explain the adaptive significance of the FMF pattern. Specifically, sex seems to be determined by the amount of development that occurs above and below the TPiv during the thermosensitive period (Georges *et al.* 1994), with high temperature resulting in a short thermosensitive period (Carter *et al.* 2018, 2019b; Massey *et al.* 2019). Temperatures below the lower TPiv have low developmental leverage and hence have a relatively weak influence on sex. Selection maintaining the lower TPiv is therefore relatively weak, and the lower TPiv may be lost (or functionally so (Schwarzkopf & Brooks 1985; Janzen 2008)) without a major influence on primary sex ratios, leaving the MF pattern of cool males and warm females.

However, if the MF pattern evolves through both the thermal limits of viability and *changes* in male-

determining temperatures (e.g., Deeming and Ferguson 1989; Ewert and Nelson 1991; Ewert et al. 2004), then the MF pattern requires more thorough dissection. The alignment of the MF pattern with Mighty Males requires that assumptions be made with respect to the position of TPiv along the axis of performance. I suggest that TPiv should occur at a temperature that results in approximately average fitness for both sexes (Fig. 3b), but critically, fitness should decline with temperature at a faster rate above TPiv (i.e., female-producing temperatures) vs below TPiv. The reasoning is that male-producing temperatures should reflect the peak of the performance curve, where a unit change in temperature has little influences on fitness, whereas female-producing temperatures should represent the shoulder of the curve, where fitness changes rapidly with temperature. Therefore, performance metrics should be relatively invariant with respect to temperature in the range of male-producing temperatures (i.e, slightly cooler than TPiv), although male-producing temperatures that are far cooler than TPiv may result in a decrease in performance.

Thermal performance and the MF and FMF patterns

The Mighty Males hypothesis, if correct, stands to provide insight into the evolution of thermal stress and thermal limits. This is because a main prediction of the hypothesis is that parameters associated with TSD (e.g., TPiv, the range of male-producing temperatures, etc) evolve so that males are produced in thermal environments that impart relatively high-quality phenotypes. Here, I summarize aspects of thermal performance theory that are relevant to Mighty Males, as well as key research on whether ecologically relevant incubation environments regularly experience heat stress.

Thermal performance curves (TPCs) characterize the relationship between performance and temperature (Huey & Stevenson 1979). Biochemical constraints dictate that the shape of TPCs are typically Gaussian and left skewed, and a key characteristic of TPCs is that they predict a rapid decrease in performance at high temperature (Schoolfield *et al.* 1981; Kingsolver 2009; Amarasekare & Johnson 2017). A consequences of TPC asymmetry is that temperatures higher than the optimal temperature for performance depress fitness more than an equivalent temperature displacement below the optimal temperature (Martin & Huey 2008). Thermoregulatory behaviour should therefore evolve such that mean body temperature is lower than the temperature that maximizes performance, as organisms do not thermoregulate with perfect accuracy, and overshooting the optimal temperature has relatively strong and negative fitness consequences (Martin & Huey 2008). For reptiles with TSD, capacity for embryonic thermoregulation exists (Ye *et al.* 2019), but embryos cannot physically displace themselves, and so thermoregulation is unlikely to result in widespread avoidance thermally stressful environments (Telemeco *et al.* 2016). The key point here is that avoidance of heat stress has a large influence on the evolution of thermoregulation, such that avoidance of heat stress can be considered evolutionarily important (Martin & Huey 2008). Given that embryos cannot move, they are generally far more susceptible than adults to the negative fitness consequences associated with heat stress.

There are many specific examples of how exposure to hot incubation environments results in low-quality phenotypes, and through a variety of pathways. For instance, exposure to extreme heat, but not extreme cold, during natural incubation results in hatchling shell deformations in wild *Chyrsemys picta* ('extreme' defined as ±2SD from grand mean incubation temperature over two years), and the deformations themselves seem to have negative fitness consequences (Telemeco *et al.* 2013). Similarly, warm incubation temperatures supress the innate immune response of hatchlings in two distantly related turtles species, whereas cool temperatures enhance immune response (Freedberg *et al.* 2008; Dang *et al.* 2015). It is also possible that elevated embryo metabolism may not be matched by increased oxygen supply in reptiles incubated at high temperatures (Hall & Warner 2019), such that negative fitness consequences may arise in part through oxygen deprivation. As a final example, high and constant incubation temperature is also associated with small size at hatchling (Warner et al. in press; Packard et al. 1987*b* , 1988; Janzen and Morjan 2002), likely because temperature has stronger effect on development than on growth at all life stages (Forster *et al.* 2011), and small size tends to be associated with lower fitness in juveniles and adults (Rollinson & Rowe 2015; Armstrong *et al.* 2017). In sum, there are a variety of ways in which hot environments can decrease phenotypic quality.

More generally, evidence of the stress imparted by high temperature arises in the existence of heat-shock

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proteins (HSPs). HSPs are a broadly conserved group of molecular chaperones designed to buffer the impact of heat stress on phenotypes (Sørensen *et al.* 2003), for which there is no known equivalent for cold stress (Sinclair & Roberts 2005). Both heat stress and/or the overexpression of heat-shock proteins have subsequent deleterious and long-term effects on performance, including development and survival, acting through a variety of phenotypic pathways (Feder & Hofmann 1999; Kingsolver & Woods 2016). Thus, one simple test of Mighty Males is to assess whether thermal stress is more likely under female-producing conditions, by testing whether the expression of heat shock proteins is positively associated with female sex under environmentally relevant conditions. Critically, testing this prediction should also be done in concert with exploring the range of incubation temperatures in wild nests in order to estimate environmentally-relevant temperatures. For instance, in some populations, embryos of FMF populations rarely experiences temperatures beyond the upper TPiv (e.g., Warner & Shine 2008a; Rollinson *et al.* 2018); in other FMF populations, temperatures below the lower TPiv are rare (Janzen 2008). Indeed, a broad spatial and temporal characterization of incubation environments is necessary to estimate environmentally relevant temperatures (e.g., Carter *et al.* 2018; Francis *et al.* 2019).

Although heat stress is generally expected at high temperature, embryonic thermal performance will ultimately adapt to the thermal environment, such that ecological context is required understand thermal stress and thermal limits. The Mighty Males hypothesis generates at least two predictions will arise from local adaptations of embryos to the thermal environment. The first deals specifically with FMF species. The logic of the prediction arises from the theoretical expectation of a trade-off between TPC height and breadth (Gilchrist 1995), such that in seasonal environments, local adaptation of TPCs will result in relatively platykurtotic TPCs centered on low mean temperatures (e.g., Fig. 4a,b). This reflects thermal adaptation to a relatively unpredictable environment that features both seasonal variation in thermal means, and pronounced diurnal thermal fluctuation of a magnitude that varies seasonally (Amarasekare & Johnson 2017; Francis *et al.* 2019). In other words, seasonality favours a form of TPC evolution where individuals are relatively tolerant of, and adapted to, a wide range of temperatures; hence "good" thermal environments for TSD species feature a wide range of incubation temperatures. This prediction is, therefore, that the range of male-producing temperatures will be positively associated with the degree of temperature variation inherent in the environment (Fig. 4a,b), or more specifically with the range of incubation temperatures experienced by the average embryo. Indirect support for this prediction is provided by Ewert et al. (2004), whose data suggest that the range of male-producing temperatures was positively associated with latitude in an FMF turtle across six populations, as is generally expected under Mighty Males (Fig. 4c). A quantitative test of this prediction is nevertheless warranted, as Ewert et al. (2004) focus on latitude and not variance in the average incubation environment. Unfortunately, it is not intuitive how thermal adaption to seasonality would influence TSD parameters in MF species under Mighty Males, precluding a similar prediction for MF species. In any event, recent evidence suggests that TSD parameters are not strongly related to latitude or longitude in at least one MF species (Carter *et al.* 2019a), although variance of incubation temperature was not explored in this study.

The second prediction of Mighty Males under local adaptation to the thermal environment is that females should suffer greater mortality than males, especially at the egg and hatchling life stages. The logic is that Mighty Males predicts TPiv to exhibit a correlated evolution with thermal performance and thermal tolerance, specifically so that TPiv marks the departure from favourable to unfavourable thermal environments that impart low-quality phenotypes. The prediction may be difficult to test at the adult stage, as viability selection on adults tends to be relatively weak in nature in the first place (Kingsolver *et al.* 2001, 2012), and females that are most strongly affected by thermal stress will die either before hatching or shortly thereafter, leaving females with relatively subtle phenotypic effects to survive until adulthood. Indeed, the only study to my knowledge to examine survival differences between the sexes of adult turtles found no difference (Chaloupka & Limpus 2005). However, this prediction should be straightforward to test at the egg and hatchling stage, where viability selection tends to be stronger (Rollinson & Rowe 2015). The specific expectation under constant incubation conditions is that fitness (e.g., embryonic survival) should depreciate relatively rapidly when embryos are incubated above vs below (upper) TPiv. Under fluctuating thermal conditions, a similar

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prediction for MF species is that fitness (e.g., survival or embryonic deformity rate) should be positively associated with the extent of female-biased sex ratios. The recent publication of a comprehensive database on phenotypic outcomes of reptilian incubation will facilitate tests of this prediction (Noble *et al.* 2018b, a).

The FM and FMF patterns in squamates and the tuatara

Several elegant studies have shown that squamates can exhibit intraspecific differences in sex determining mechanisms, specifically in GSD vs TSD (Pen *et al.* 2010; Holleley *et al.* 2015). The ecology and evolution of TSD may therefore be more complex in squamates than in turtles and crocodilians, but I nevertheless believe that Mighty Males can provide insight in to TSD in squamate reptiles. The biggest challenge is to reconcile Mighty Males with the occurrence of the FM pattern in squamates and the tuatara. This is because FM is inconsistent with the assumption that warm temperatures produce low-quality phenotypes.

There are several reasons that the MF does not undermine the Mighty Males hypothesis. The first reason is that the FM pattern is extremely rare (Mitchell *et al.* 2006), and arguably, FM may not even exist. A majority of TSD species originally described as FM have subsequently been recategorized as FMF when a wider range of incubation temperatures were tested (Lang & Andrews 1994; Godfrey *et al.* 2003). The recategorization is extensive, and may even include *Agama agama*(Steele *et al.* 2018), the species that gave rise to the study of TSD in the first place. In fact, I am only aware of one species, the tuatara, where evidence of FM has been recently defended (Mitchell*et al.* 2006); unfortunately, the conservation status of the tuatara makes further investigation difficult. Future investigation of FM in squamates may therefore uncover evidence of FMF, such that the Mighty Males hypothesis its various predictions can be explored.

Assuming the FM pattern is real but very rare, then the adaptive explanation for TSD in the tuatara and squamates may be different than in turtles and crocodilians. In other words, Mighty Males (FMF, and MF) would apply to turtles, crocodilians, and other explanations for TSD would apply to FM squamates and the tuatara. Existing explanations for TSD in short-lived FM squamates and short-lived FM fish rely on the timing of reproduction, where females are produced under cool temperatures early in the growing season so that growth and hence fecundity is maximized during a short life cycle (e.g., Conover 1984; Warner & Shine 2005; Pen *et al.* 2010). Such a mechanism is very unlikely in turtles or crocodilians because of late age at maturity and incredible variation in growth rates (Armstrong *et al.* 2017; Congdon *et al.* 2018). Divergent adaptive explanations for TSD could arise if TSD evolved independently in different reptile lineages. Notably, the tuatara and squamates are sister groups (Rest *et al.*2003), whereas turtles and Archosaurs, which includes crocodilians, comprise a different sister group (Crawford *et al.* 2012). An intriguing possibility, therefore, is that the ancestor of turtles and crocodilians exhibited TSD, whereas the ancestor of squamates exhibited GSD, such that TSD evolved only recently in the tuatara and a few squamate lineages. Different adaptive explanations for TSD might then become likely in these different groups. One study supports the notion of divergent ancestral sex-determining mechanisms in major reptile clades (Janzen & Krenz 2004), but more recent evidence suggests that TSD is ancestral in all reptile groups, and that GSD is derived (Pokorná & Kratochvíl 2009; Gamble *et al.* 2015; Sabath *et al.*2016). This second explanation for the FM pattern, then, seems to depend on transitions to GSD followed by reversions to TSD in squamates; there is currently no evidence for this (see also Holleley *et al.*2015), but we know that sex determining mechanism are, at least, highly labile in some reptiles (Gamble *et al.* 2015). Alternatively, millions of years of independent evolution of TSD in turtles and crocodilians vs squamates and the tuatara may have plausibly resulted in differences in the adaptive function of TSD, even if TSD is ancestral to both groups.

I emphasize that it is only the rare FM pattern that is difficult to reconcile with Mighty Males, whereas the FMF pattern observed in most lizards can and should be explored under the lens of Mighty Males. For instance, the leopard gecko (*Eublepharis macularius*) features an FMF pattern, although males and females are produced over a broader range of temperature than in many other species, allowing temperature and sex to be decoupled without hormonal manipulation. Females have a determinate clutch size of two eggs, and female fitness is not very sensitive to temperature. Males experience high intra-sex aggression, presumably allowing them to secure mating opportunities, and consistent with Mighty Males, males produced at intermediate incubation temperature win significantly more aggressive encounters (reviewed by Rhen &

Crews 2001). Similarly, lifetime reproductive success for an FMF lizard, *A. muricatus* , was greatest for males produced at intermediate temperatures, as opposed to sex-reversed males produced under high and low temperatures, whereas performance of naturally-produced females was inconsistent across years (Warner & Shine 2008b). This provides some evidence that male-producing temperatures provide the greatest lifetime reproductive success, at least for males. In sum, in both of these FMF squamate examples, fitness interacts with temperature and sex in a manner that is broadly consistent with Mighty Males, underlining that the explanatory scope of Mighty Males is not necessarily limited to turtles and crocodilians.

Similarities to existing theory

Two existing hypotheses, both conceived in the Charnov-Bull framework, exhibit some similarities to Mighty Males. The Sexual Dimorphism hypothesis (Head *et al.* 1987) proposes that incubation temperature affects post-hatching growth, and TSD evolves to maximize post-hatching growth rate of the larger sex. The evidence does not support this hypothesis broadly in reptiles (Janzen & Paukstis 1991a, b), although male-producing temperatures enhance growth rate relative to female-producing temperatures in some turtles and crocodilians with male-biased size dimorphism, potentially allowing males to grow large and guard a harem of females (Deeming *et al.* 1988; Deeming & Ferguson 1989; Bobyn & Brooks 1994a; Rhen & Lang 1994). The Mighty Males hypothesis is formulated in a spirit that is similar to the Sexual Dimorphism hypothesis, but the former is more general. Specifically, Mighty Males recognizes that males are generally mate-limited and experience stronger sexual selection than females regardless of size dimorphism (Singh & Punzalan 2018). Critically, Mighty Males does not predict that growth rate of males is always greater than that of females. Although the quality of the embryonic environment may affect growth rate, growth rate may also be associated with asymptotic size in a sex-specific manner (Le Galliard *et al.* 2005), and size dimorphism can evolve for reasons that are divorced from the focal hypothesis (De Lisle & Rowe 2013, 2015). Thus, evaluation of Mighty Males with respect to juvenile growth rate must be done by decoupling sex and environment.

The Sex-Specific Survival to Maturity hypothesis proposes that TSD will evolve when incubation temperature affects juvenile survival equivalently for males and females, and the sexes differ in age at maturity (Schwanz *et al.* 2016). A sex by environment interaction for fitness occurs when the favourable (high survival) incubation environment produces the sex maturing at a later age, and the less favourable environment (low survival) produces the sex maturing earlier. This idea is similar to Mighty Males in that incubation environments vary in quality and the incubation environment affects the quality of male and female phenotypes equally; however, Mighty Males emphasizes fertility at adulthood, whereas Sex-Specific Survival to Maturity emphasizes its namesake. The generality of Sex-Specific Survival to Maturity may be limited because there is no systematic pattern in sex-specific maturational ages across turtle species (Berry & Shine 1980; Bókony *et al.* 2019) and possibly crocodilians as well (Cox *et al.* 2007). For the hypothesis to be valid, female-producing temperatures would represent high-quality (high-survival) incubation environments in female-size (age) dimorphic species, and male-producing temperatures must be high-quality (high-survival) environments in male-size (age) dimorphic species. So, while the Sex-Specific Survival to Maturity hypothesis should be more broadly tested, the underlying assumptions are perhaps more complex and less supported than in Mighty Males. Indeed, Mighty Males makes no assumption regarding sexual differences in size or age, and under Mighty Males, high-quality incubation environments are always those that produce males.

Explaining sex beyond TSD

If Mighty Males is correct, then sex determination occurs because of the effect of the environment on phenotypic quality. Temperature just happens to be a pervasive agent in the environment that has a profound influence on phenotype. But if sex determination occurs because of environmental effects on phenotypic quality, then it seems possible that environmental factors other than temperature should affect sex in TSD species. Or, other environmental factors might at least modify how temperature affects sex, ultimately ensuring that high-quality phenotypes are linked to male production. In fact, there is good evidence that factors other than temperature affect sex ratios in "TSD" species. A strength of Mighty Males is that it can explain multiple forms of ESD operating within a "TSD" group or population.

In two TSD species of turtle (*Trachemys* and *Graptemys*), incubation of turtle eggs under low O_2 and/or high CO_2 concentrations simultaneously increases mortality (suggesting negative phenotypic effects) and results in a dramatic overproduction of females (Etchberger *et al.* 2002). This suggests multiple ESD mechanisms in the same species, and the finding is ecologically relevant as elevated CO_2 and depressed O_2 concentrations can occur naturally in turtle nests through embryonic and microbial respiration, coupled with low gas exchange with the surface e.g., under wet conditions (Ackerman 1980; Ackerman & Lott 2004). If we accept that Mighty Males acts through phenotypic quality and not necessarily temperature alone, then the overproduction of females in low O_2 environments is not surprising, given the low phenotypic quality of individuals that likely arise from stressful and oxygen deprived environments. As another example, moisture in wild reptile nests affects fitness-related traits, with moist nests tending to produce larger and more robust offspring (reviewed by Packard 1999). Experimental addition of water to turtle embryos during the thermosensitive period results in male-biased sex ratios (LeBlanc & Wibbels 2009; Lolavar & Wyneken 2017), suggesting that moisture affects both phenotype and sex in some turtle species, but not all turtle species (Paukstis *et al.* 1984; Packard *et al.* 1989), in the direction predicted by Mighty Males. More generally, a multiplicity of ESD mechanisms is not unexpected under Mighty Males, and the possibility of multiple ESD mechanisms under TSD should be investigated more broadly (Warner *et al.* 2017).

Finally, in addition to accommodating multiple forms of ESD operating within 'TSD" species, Mighty Males can also explain forms of TSD that are sensitive to the maternal environment. For example, Maternal effects on TPiv occur in at least two MF species of freshwater turtle, where embryos incubated at the population TPiv are more likely to develop into females when eggs are laid late in the season, compared to eggs laid early in the season. Thus a decline in TPiv across the season is inferred, which is observed under constant conditions in the lab, and this decline affects the sex ratio under fluctuating temperatures in the field (Bowden *et al.* 2000; Carter *et al.* 2017). Hormonal differences provide a proximate reason for the greater propensity of embryos to become female as the season progresses, but no ultimate reason has been proposed (Bowden *et al.* 2000). Life-history theory and a variety of data suggest that individuals in low condition nest later in reproductive season (Rowe *et al.* 1994), laying fewer eggs that are relatively poorly provisioned (Harms *et al.*2005). In turtles, for instance, relatively young females nest late in the season, and their offspring have lower survival than offspring of the same size that are produced by older females (Paitz *et al.*2007; Rollinson & Brooks 2008). Female quality may be the ultimate driver of changes TPiv across the reproductive season, with an overproduction of females reflecting the low-quality offspring produced by mothers in relatively poor condition (Trivers & Willard 1973). Notably, this would not be the first example of maternal effects modifying the extent of TSD in a reptile (Radder *et al.* 2009), but it is one that involves modification of intraspecific TSD patterns by maternal effects in a manner potentially consistent with Mighty Males.

Conclusion

The Mighty Males hypothesis has several attractive properties. First and foremost, it is simple, relying on only a few assumptions that are well supported. Second, a common difficulty when applying the Bull-Charnov Model to a given situation is that sex-specific benefits can be argued from the perspective of advantaging males, or advantaging females, even within the same species (e.g., compare Harlow and Taylor 2000; Warner and Shine 2005). The Mighty Males hypothesis generates the same or very similar predictions for every species, some of which already have good support, and therefore the hypothesis can be tested unambiguously. Finally, Mighty Males also implies that environmental factors other than temperature may affect sex determination, even in species traditionally known to exhibit TSD. The hypothesis may therefore help explain why species simultaneously exhibit TSD and other forms of ESD as well. More broadly, I suggest that our knowledge of TSD is less complete than is currently appreciated, and I hope in earnest that the present essay spurs further investigation into TSD and other forms of ESD in reptiles and elsewhere, perhaps guided by some of the ideas that fall naturally out of the Mighty Males hypothesis.

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Figure 1: The three patterns of sex determination in reptiles. (a) FMF is ancestral and is found in turtles, crocodilians, and lizards, (b) MF is derived and found in turtles, (c) FM is derived and found in tuataras and lizards. TPiv is the pivotal temperature, which is the temperature where the sex ratio is 50:50 under constant temperature incubation.

Figure 2 : Histogram of fitness distribution for (a) males and (b) females at sexual maturity.

Figure 3 : The relationship between environmental quality and expected fitness for males and females. (a) Visualization of the classic Trivers-Willard hypothesis. Maternal quality has a differential influence on fitness of sons and daughters (assuming quality is passed across generations), with the switch point representing the value of x where mothers should switch bias in sex allocation. (b, c) The Mighty Males hypothesis is similar to Trivers-Willard, but incubation temperature influences condition, rather than properties of the mother. In panels (a) FMF and (b) MF, males should be produced under the conditions that maximize fitness for both sexes, as males stand to gain more from good incubation environments, and to lose more bad incubation environments. In panel (b) the lower TPiv (light grey) is meant to depict the fact that females can be produced under extremely low temperatures in MF species (Schwarzkopf & Brooks 1985).

Figure 4 : The evolution of thermal performance gives rise to embryonic adaptation that is related to thermal variability, which predicts the extent to which phenotypes are susceptible to thermal stress. Mighty Males predicts that the range of male-producing temperatures will be wider when embryos are adapted to environments that feature regular incursions into thermal extremes. (a) The evolution of thermal performance in a seasonal environment (e.g., northern latitude), with the histogram of environmentally relevant temperature (and mean embryonic temperature, To) in the background. Phenotypes experience thermal extremes and a wide range of incubation temperature. Males are produced over a relatively wide-range of incubation temperatures as individuals evolve to be robust under thermal variability. (b) The evolution of thermal performance in an aseasonal environment (e.g., tropics). Males are produced under a narrow range of temperature because embryos are less subject to thermal variability, and hence are not tolerant of thermal extremes. (c) Redrawn from Ewert et al. (2003). Thermal reaction norms for sex were measured at constant temperatures in snapping turtle (*Chelydra serpentina*) populations from Minnesota (MN), Michigan (MI), Indiana (IN), Arkansas and Louisiana (AR-LA), western Florida (W.FL) and peninsular Florida (P.FL). Note that the range of male-producing temperatures seems to decline with approximate latitude, to the point that no temperature produces 100% males in more southern areas.

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