

Chapter 12

Causes and Consequences of Evolutionary Transitions in the Level of Phenotypic Plasticity of Reptilian Sex Determination



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Abstract Reptiles exhibit a remarkable variety of mechanisms by which the sexual fate of developing individuals is decided, ranging from systems of extreme phenotypic plasticity dependent on incubation temperature (TSD) to others of extreme canalization under the control of sex chromosomes (GSD). Our understanding of the extent of this diversity is growing, as is the insight about its molecular basis (genetic and epigenetic). However, further research is still overdue to fully decipher the causes and consequences of the independent and frequent transitions between TSD and GSD in reptile lineages. For instance, theoretically, it may be expected that TSD lineages suffer higher extinction because TSD may result in highly biased sex ratios that are detrimental for population dynamics, while sex chromosomes may promote higher speciation rates. Yet, support from empirical studies is conflicting and instead, some evidence points to longevity as an important mediator for the evolution of sex-determining mechanisms. While much effort has been devoted to test whether TSD or GSD is adaptive in various lineages, one of the most exciting areas of inquiry today relates to the increasing reports of intermediate systems of sex determination in reptiles (GSD + TSD = GSD susceptible to thermally induced sex reversal) as they shed critical light on the constraints (or lack thereof) for the evolution of sex determination in this group. The flourish of genomic approaches applied to the study of sex determination along with the refinement of phylogenetic comparative methods are also enabling the reconstruction of the enigmatic micro- and macroevolutionary history of reptilian sex determination.

12.1 Introduction

Sexually reproducing organisms with separate sexes across the tree of life vary wildly in the way they commit to the male or female developmental fate (Bachtrog et al. 2014; Bull 1983). These sex-determining mechanisms (SDMs) range from

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systems with a high degree of phenotypic plasticity to others with extreme developmental canalization, as occurs in vertebrates (Tree of Sex Consortium et al. 2014; Valenzuela and Lance 2004). Among amniotes, reptiles are particularly labile in the mechanisms they employ to determine sex. At one end of the plasticity continuum, we find reptilian systems that trigger sex-specific development according to the environmental temperature experienced during embryogenesis (temperature-dependent sex determination—TSD) in the absence of consistent genotypic differences between the sexes (thermal polyphenism), while in the other end, we find canalized systems where the trigger is primarily the individual's genotypic constitution (genotypic sex determination—GSD) such as occurs in chromosomal sex determination (Valenzuela et al. 2003, 2014; Sarre et al. 2004). Systems with intermediate levels of canalization/environmental plasticity lay between these two extremes. Reports of intermediate mechanisms in reptiles are increasing and include some well-documented cases where temperature overrides the genotypic signal from sex chromosomes in nature (Shine et al. 2002; Holleley et al. 2015; Radder et al. 2008), while other instances of potential co-occurrence of TSD and GSD have been refuted out empirically (Valenzuela et al. 2014; Mu et al. 2015). The causes and consequences of this remarkable diversity and the turnovers in SDM remain incompletely understood. Studies of sex determination encompass a variety of disciplines and levels of organization. They range from ecological research about the context where various SDMs are found and their effect on sex ratio production and population dynamics, to functional research about the molecular underpinnings of sexual development, to phylogenetic analysis of SDM prevalence and turnover, to theoretical studies about potential drivers of SDM evolution such as sex allocation, sexual selection, or genomic conflict. Here I review our current knowledge about transitions between TSD and GSD in reptiles, models about the relative advantages and disadvantages of each SDM, and whether they are supported by evidence from empirical tests or not.

12.2 Reptilian Sex Determination

A recent explosion of studies of sex determination facilitated by a combination of classic and modern molecular approaches reveals that TSD and GSD coexist within some vertebrate orders, families, and even species, as is the case in reptiles (Tree of Sex Consortium et al. 2014; Pokorná et al. 2011, 2014a, b; Ota et al. 1992; Gamble 2010; Gamble et al. 2014, 2015; Trifonov et al. 2011; Koubová et al. 2014; Schmid et al. 2014; Matsubara et al. 2013; Matsubara et al. 2014; Rovatsos et al. 2014a, b; Sulandari et al. 2014; Badenhorst et al. 2013; Montiel et al. 2017), yet some lineages are surprisingly conserved, such as mammals and birds, while the well-accepted conservation of snakes was recently debunked (Gamble et al. 2017) (Fig. 12.1). Further efforts to identify the SDM in additional reptiles are still warranted because the current taxonomic coverage of all studies combined (Tree of Sex Consortium et al. 2014) encompasses only a fraction of the existing reptiles, obscuring the

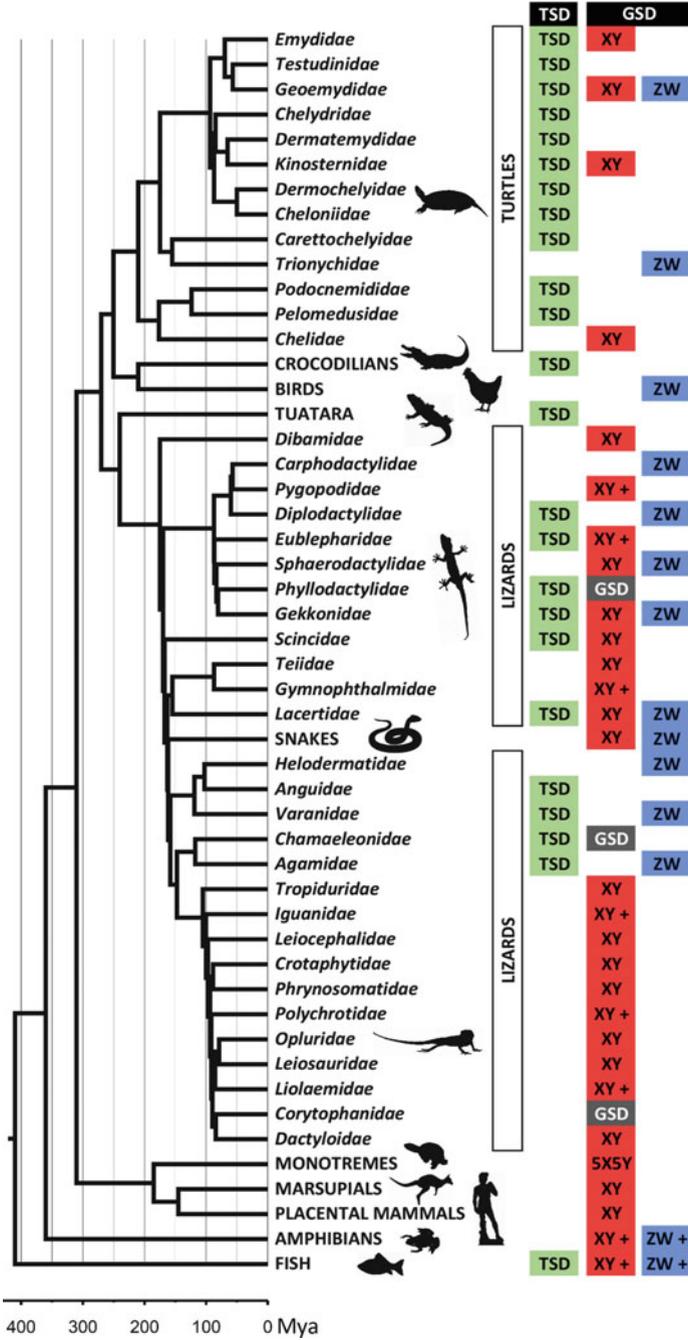


Fig. 12.1 Sex-determining mechanism in reptilian families and other vertebrates. Phylogeny and sex determination information are based on Gamble et al. (2017), Sabath et al. (2016), Chiari et al. (2012), and Jones et al. (2013)

evolutionary history of SDM and correlated traits in this group (Sabath et al. 2016). Interestingly, while TSD reaction norms in turtles commonly span temperatures that produce 100% males and 100% females (Ewert et al. 2004), this is more rarely the case in lizards (Harlow 2004) and crocodylians (Deeming 2004) where fewer species produce 100% males at any given temperature (but the nature of this constraint remains unknown). Several studies now indicate that TSD is the likely ancestral state in reptiles from which GSD evolved independently multiple times, with reversals occurring more often in squamates than in turtles (Sabath et al. 2016; Pokorná and Kratochvíl 2009; Valenzuela and Adams 2011), as detailed below (see section on SDM transitions in reptiles).

The molecular basis of sex determination in reptiles is also receiving considerable attention. Work in this area has concentrated in identifying reptilian homologs of genes known to be involved in sexual development in mammals and birds (Eggers et al. 2014; Smith 2010) and studying their expression patterns during reptilian gonadal development. More recent studies have interrogated reptilian genomes in an unbiased fashion using transcriptomics and illuminated the full composition of the gene network that regulates gonadal formation in turtles (Czerwinski et al. 2016; Radhakrishnan et al. 2017) and alligator (Yatsu et al. 2016). Because of the fascination that TSD has attracted since its discovery half a century ago (Charnier 1966), most of these molecular developmental studies have examined TSD reptiles (turtles, crocodylians, lizards) [e.g., (Czerwinski et al. 2016; Radhakrishnan et al. 2017; Yatsu et al. 2016; Bieser and Wibbels 2014; Rhen and Schroeder 2010; Barske and Capel 2010; Valenzuela et al. 2013; Schroeder et al. 2016; Shoemaker and Crews 2009; Ramsey and Crews 2009; Willingham et al. 2000; Paitz and Bowden 2013; Endo et al. 2008; Choudhary et al. 2000; Xin et al. 2014; Parsley et al. 2014; Inamdar et al. 2015; Parrott et al. 2014; Janes et al. 2013; Smith and Joss 1994; Smith et al. 1995), with the exception of *Apalone* softshell turtles, the only exclusively GSD reptile whose primary sexual development has been studied thus far in this vein (Valenzuela 2008a, b, 2010a; Valenzuela et al. 2006; Valenzuela and Shikano 2007)]. Combined, these efforts indicate that all the components of this regulatory gene network that have been investigated exist in reptiles (Fig. 12.2) with the notable exception of the *Sry* gene which is unique to eutherian mammals (Wallis et al. 2008). Despite the overall conservation in the composition of this network (Graves and Peichel 2010; Cutting et al. 2013), expression patterns of common elements differ within reptiles and among vertebrates (Valenzuela et al. 2013; Cutting et al. 2013). The elegant molecular architecture of sex determination/differentiation can be tipped off-balance by environmental insults as is the case of contaminants such as endocrine disruptors (EDCs) which can yield suboptimal phenotypes both in TSD and GSD reptiles (reviewed in Mizoguchi and Valenzuela 2016). Importantly, multiple elements in this network exhibit plastic responses to environmental temperature during gonadal formation in TSD taxa (differential transcription, translation, or protein activity by temperature) at various developmental stages (reviewed in Rhen and Schroeder 2010; Merchant-Larios et al. 2010). And some responses occur even before the canonical thermosensitive period, i.e., the time window when temperature exerts the

(Montiel et al. 2017; Montiel et al. 2016), which is intriguing because *Wtl* has been postulated as an upstream TSD candidate in the turtle developmental cascade that may play a key role in the early conversion of incubation temperature to sex-specific development, or it may be near the TSD factor that does so (Valenzuela 2008a). Moreover, the evolution of these turtle GSD systems involved chromosomal inversions that encompassed *Dmrt1* or *Wtl* (Montiel et al. 2017; Kawagoshi et al. 2009, 2012, 2014), a likely decisive event for the molecular evolution steps that might have led to GSD evolution and the subsequent divergence of these sex chromosomes themselves (Bachtrog et al. 2011; Charlesworth 2002). Importantly, because vestigial thermal sensitivity is sometimes retained in elements of this regulatory network by taxa with a derived GSD system (Valenzuela 2008a, b), it is plausible that transitions from GSD to TSD could also be facilitated via the co-option of some of those elements in certain lineages, but this remains an untested hypothesis.

12.3 SDM Transitions in Reptiles and What Drives Them

Earlier phylogenetic analyses found equivocal support for the ancestral SDM in reptiles when using family level (Organ and Janes 2008) or restricted sampling approaches (Janzen and Krenz 2004), but later species-level studies reconstruct TSD as the likely ancestral state in squamates (Sabath et al. 2016; Pokorná and Kratochvíl 2009), turtles (Sabath et al. 2016; Valenzuela and Adams 2011), and perhaps even amniotes (Pokorná and Kratochvíl 2016). Thus, GSD appears to have evolved multiple times in diverse reptilian lineages. Reversals back to TSD are more common in squamates, specifically in lizards (Sabath et al. 2016; Pokorná and Kratochvíl 2016), and only rare (if ever present) in turtles (Sabath et al. 2016; Valenzuela and Adams 2011; Litterman et al. 2018). Consistently, the transition rates among SDMs varies in turtles and lizards, the two reptilian groups with labile sex determination, with GSD-to-TSD transitions being more prevalent than TSD-to-GSD transitions in lizards, whereas in turtles transitions are more rare and do not differ in direction (Sabath et al. 2016). Thus, turtles appear to retain their ancestral TSD state, whereas lizards have given up TSD for GSD fairly easily over evolutionary time.

But what are the ultimate drivers of these SDM transitions we observe in nature and that take place via the molecular changes described above, or their constraints? Are SDM turnover events random or adaptive responses to selective pressures? These questions have been the focus of extensive studies, both theoretical and empirical, and remain an area of active inquiry and debate, including in reptiles. It is clear that SDMs affect sex ratio production, and in doing so, they can alter effective population sizes and the rate of loss of genetic variation that are linked to extinction probabilities (Bachtrog et al. 2014; Valenzuela and Lance 2004; Girondot et al. 2004; Bessa-Gomes et al. 2004). Namely, because TSD produces sex ratios according to the environmental temperature experienced during development, the potential disadvantages of TSD are easily envisioned. Specifically, TSD populations could produce highly skewed sex ratios due to climatic variation which can cause

population extinction and thus favor the evolution of compensating mechanisms to balance the sex ratios, including the evolution of a GSD system (Bull 1980). This matches the theoretical expectation that balanced sex ratios are the evolutionary stable strategy when the cost of producing males and females is the same (Fisher 1930). Also consistent with this idea, turtle lineages within which SDM turnover took place during >210 My of evolution split from their sister clades near peaks of global temperature that may have led to directionally biased sex ratios which were counterbalanced in some lineages by the evolution of GSD (Valenzuela and Adams 2011). The production of intersexes could be another potential detrimental by-product of TSD (Bull 1981), but intersexuality appears to be transient if present at all, at least in turtles (Pieau et al. 1998; Girondot et al. 1998), and simultaneous hermaphroditism is unknown in reptiles (Leonard 2013).

Life histories can play a significant role in triggering or impeding SDM transitions. For instance, the discrepancies in the rates of SDM transitions observed in turtles and lizards are linked to differences in longevity between these two groups (Sabath et al. 2016) which is expected because life span can render TSD and GSD adaptive, maladaptive, or neutral (Bull and Bulmer 1989; Valenzuela 2004; Freedberg and Debenport 2014; Schwanz and Proulx 2008). Namely, lizards abandon TSD and replace it with GSD more often than turtles likely because their generally shorter life span makes them vulnerable to highly skewed sex ratios produced by the vagaries of the environmental temperature (Sabath et al. 2016). And in fact, TSD turtles and lizards evolve toward greater life span compared to their GSD counterparts, an effect that is more pronounced in turtles than in lizards (Sabath et al. 2016). This also implies that given turtle longevity, TSD may be more of a neutral trait which is retained in this group because it works just as well as GSD (Bull 1980; Valenzuela 2004; Girondot and Pieau 1999). On the contrary, given the shorter life span of lizards, TSD in this group, when present, must be maintained by quite strong selective forces, or a transition to GSD would occur when TSD is detrimental. But how could TSD be adaptive at all?

Substantial effort has been devoted to study the potential adaptive advantage of environmental sex determination in general and of TSD in reptiles in particular, and how it could trigger the shift from GSD. One possibility is that biased sex ratios might be favored and TSD provides an adaptive plasticity mechanism to accomplish just that (Shaw and Mohler 1953). Various hypotheses have been proposed to explain when biased sex ratios might be adaptive. For instance, if species are structured into smaller demes, TSD may permit the overproduction of females that would bolster population growth (Bull and Charnov 1988). However, if populations are drastically small, TSD-induced sex ratio skews can push them beyond the limit where *Alee* effects causes their extinction instead of aiding population growth (Berec et al. 2001), in which case a shift to GSD would be favored. Instead, the *Charnov-Bull* model is the most robust of the theoretical models about how biased sex ratios induced by TSD may be adaptive, and it has withstood the test of time over four decades (Charnov and Bull 1977). This model predicts that when the environmental temperature experienced during development (or a correlated variable) confers males and females a lifetime differential fitness in a way that is unpredictable by

the parents or the offspring, TSD is favored over GSD because its phenotypic plasticity affords the developing offspring the chance to differentiate into the sex that attains the highest fitness under particular environmental conditions.

The *Charnov-Bull* model does not apply to all cases ubiquitously or in the same manner. For instance, differential fitness may derive from sex-specific life histories, such as from sex-specific mortality (Burger and Zappalorti 1988), or if female quality depends on the site they are born to which they return to nest when adults (Reinhold 1998), an effect that is accentuated if males disperse more than females (Julliard 2000). But support for these alternatives is mixed. TSD was also proposed as a mechanism to induce sex-specific behavioral, morphological, or physiological effects (Tousignant and Crews 1995), or optimal growth by the larger sex in species with sexual size dimorphism because the fitness gain of the larger sex increases faster as a function of body size (via fecundity or mating success) than for the smaller sex (Ewert and Nelson 1991; Head et al. 1987). However, other factors such as temperature and resource availability post-sex determination are as important or more important in determining sexual size dimorphism in reptiles as is incubation temperature directly (Ceballos et al. 2014; Ceballos and Valenzuela 2011; Cox and Calsbeek 2009; Cox et al. 2006; Starostova et al. 2010). And further, sexual size dimorphism is independent of SDM in turtles (Ceballos et al. 2012). Nonetheless, cases where environmental temperature has an *indirect* effect on fecundity have provided the strongest evidence yet for the *Charnov-Bull* model in reptiles [*Amphibolurus muricatus* lizards (Warner and Shine 2008)], and fish [*Menidia menidia* (Conover and Heins 1987)]. In these two disparate short-lived vertebrates, the environmental temperature experienced by the developing offspring provides an indirect cue about the time left in the reproductive season before the arrival of the first winter when growth ceases and sexual maturation occurs. Individuals born earlier in the spring under colder temperatures develop as females and attain larger body sizes that afford them greater fecundity, whereas individuals born later under warmer temperatures develop into males whose fitness is not as impacted by body size. A recent theoretical model combines the sexual dimorphism hypothesis with life history effects and proposes that TSD is favored when males and females mature at different ages if temperature influences juvenile survival (albeit equally for both sexes), but when tested in turtles, this model was unsupported (Schwanz et al. 2016). It should be noted that TSD is not the only mechanism that permits sex ratio adjustments. Indeed, GSD species can alter sex ratios by a variety of means, including meiotic distorters, chromosome-specific fertilization, and sex-specific mortality, among others (Valenzuela et al. 2003; Uller and Badyaev 2009; Uller et al. 2006).

12.4 Constraints to SDM Transitions

The forces that maintain a given SDM constrain by definition the transition to an alternative SDM. For instance, as with the sexual dimorphism hypothesis, TSD could be favored if female nest site choice takes place in an egg-size-dependent

manner in a species where larger eggs produce larger hatchlings that attain higher fitness, provided that those choices also affect offspring sex (Roosenburg 1996). This explanation may not be widespread, and other maternal effects associated with egg size may be at work, such as steroid allocation (Radder et al. 2009) which is an important molecular regulator of sexual development (Fig. 12.2). Additionally, maternal control of offspring sex when coupled with offspring quality could lead to runaway sex ratio skews that would ultimately select for GSD evolution (Bull 1980). Alternatively, the inherent phenotypic plasticity of TSD may confer a pre-adaptation against climate change, particularly if genetic variation exists within and among populations for the thermal sensitivity in sex ratio production and for the molecular machinery regulating TSD sexual development (Ewert et al. 2004; Valenzuela et al. 2013; Kallimanis 2009; Escobedo-Galvan et al. 2011). This may also be facilitated by epigenetic modifications such as DNA methylation which is implicated in the molecular regulation of TSD (Navarro-Martin et al. 2011; Matsumoto et al. 2013) and has been linked to responses to environmental change (Angers et al. 2010). This scenario may also explain the lower extinction of TSD versus GSD families observed during the climate change of the Cretaceous/Paleogene transition (Escobedo-Galvan and Gonzalez-Salazar 2012; Silber et al. 2011). The reported inter- and intrapopulation variation in thermal sensitivity and the molecular regulation of sexual development (Holleley et al. 2015; Ewert et al. 2004; Valenzuela et al. 2013; Uller and Helantera 2011; Grossen et al. 2010) also speak against the idea that transitions from TSD to GSD have been prevented by phylogenetic inertia (Bull 1980). Other hypotheses postulate that TSD produces unisexual clutches as a sib-avoidance strategy to prevent inbreeding (Ewert and Nelson 1991; Burke 1993) or that the biased sex ratios observed in TSD taxa are favored by culturally inherited natal homing (Freedberg and Wade 2001). Constraints for the evolution of pure TSD systems and their implication have also been proposed (Uller and Helantera 2011; Valenzuela 2010b), but empirical tests in reptiles suggest that the TSD extreme of the SDM continuum may indeed exist (Valenzuela et al. 2014). Some of these and other hypotheses have been discussed in detail elsewhere (Valenzuela et al. 2014; Valenzuela 2004, 2010b; Uller and Helantera 2011; Beukeboom and Perrin 2014).

12.5 The Intermediate Steps of SDM Transitions

Evolutionary transitions between GSD and TSD imply that populations/species traverse intermediate states in their way from one extreme to the other of the SDM continuum (Valenzuela et al. 2003; Uller and Helantera 2011; Grossen et al. 2010; Beukeboom and Perrin 2014; Sarre et al. 2011). If “pure” GSD and TSD systems were the highest fitness peaks in the SDM optimality landscape and represent evolutionary stable states, then SDM turnover would require crossing fitness valleys where populations will suffer from lower fitness. Such scenario would make intermediate mechanisms unstable states and selection will favor the rapid transition to

the alternative fitness peak, rendering these mixed SDMs transitory (Bull 1983; Valenzuela 2004; Uller and Helantera 2011) and probably rare in nature. This hypothesis is supported by the relative scarcity of intermediate SDM thus far reported.

However, alternative SDM mechanisms or certain properties of the molecular machinery that regulate sexual development might act as facilitators for SDM turnover by raising the fitness valley that species need to traverse from one to the other extreme. Or there may be neutral paths in the fitness landscape connecting various SDMs that permit readily transitions among them (Bull 1983). Indeed, an elegant recent model proposes that when sex determination is viewed as the result of a dosage-dependent process derived from the copy number of genes carried by sex chromosomes that is also susceptible to extreme temperatures in the viability range, transitions between GSD and TSD can occur easily (including transitions between male and female heterogamety and various TSD modes) as the system responds to frequency-dependent selection for sex ratio, provided that the sex chromosomes are not highly degenerate (such that YY or WW individuals are viable) (Quinn et al. 2011). This is consistent with quantitative models of gene-by-environment interactions that look at sex determination as a population- rather than an individual-level process, and provides a framework for SDM transitions when sex reversals are induced environmentally and sex ratio selection ensues (Grossen et al. 2010).

Other routes for transitions between male and female heterogamety are discussed elsewhere and range from drift models that lead to the fixation of sex chromosomes in small populations, to models of sexually antagonistic selection and chromosome fusion (Bachtrog et al. 2011; Beukeboom and Perrin 2014; Sarre et al. 2011; Pennell et al. 2015). Thus, perhaps some of these mixed SDM systems are more ancient and evolutionarily stable than originally thought rather than transitory steps between inevitable extremes in the SDM continuum. Ancestral SDM reconstruction (Sabath et al. 2016) combined with a dated squamate phylogeny (Pyron and Burbrink 2014) indicates that the mixed SDMs that are currently well documented in reptiles, in *Pogona vitticeps* (Holleley et al. 2015) and *Bassiana duperreyi* (Radder et al. 2008) lizards, are likely 25-50 My old and appear to be evolving still (Holleley et al. 2015; Radder et al. 2008). In contrast, the youngest turtle sex chromosome system known to date from *Glyptemys insculpta* (Montiel et al. 2017) and *Glyptemys muhlenbergii* (Literman et al. 2017) is ~20 My old (Montiel et al. 2017; Literman et al. 2017). Interestingly, *Bassiana duperreyi* also exhibits an egg-size-dependent allocation of yolk hormones which permits this lizard to balance sex ratios back to 50:50 adaptively when TSD causes a sex ratio skew in certain populations (Radder et al. 2009). In general however, the current scarcity of reports of mixed SDMs may be due simply to lack of proper sampling, because (a) a large proportion of species remain unexamined, (b) because sex chromosomes may be cryptic (Badenhorst et al. 2013; Ezaz et al. 2006), and (c) because search for mixed SDMs may not have been the target of many studies such and they may have been overlooked.

12.6 Consequences of SDM Transitions

The evolution of some traits is associated with transitions in SDM, but in some cases, it can be difficult to distinguish whether they are the cause or the consequences of SDM turnover. For instance, because of the effect of sex ratios on population dynamics, an association between SDM transitions and speciation or extinction is to be expected (Organ et al. 2009; Janzen 1994; Neuwald and Valenzuela 2011; Haldane 1922). But while the transition from TSD to GSD was proposed as a trigger of speciation in extinct lineages of marine reptiles (Organ et al. 2009), other studies found no association between diversification and SDM in Sauropsida (the clade of reptiles plus birds) (Organ and Janes 2008), nor on turtles, lizards, or squamates individually (Sabath et al. 2016). SDMs also correlate with adult sex ratio such that species with female heterogamety (ZZ/ZW) exhibit stronger male-bias than those with male heterogamety (XX/XY), a difference that can alter the demography of populations (Pipoly et al. 2015). On the other hand, SDM shifts in turtles correlate with an increase in chromosome reshuffling that alters diploid number but it is unclear if one occurs first and triggers the other (Valenzuela and Adams 2011). Transitions from TSD to GSD in reptiles involve the evolution of sex chromosomes (the most common GSD mechanism in animals), which in itself can have profound consequences for the evolution of sexual dimorphism, the onset of sexual dimorphism at earlier developmental stages, and its elaboration via the accumulation of sexually antagonistic genes in the heterogametic sex chromosome, compared to TSD taxa (Valenzuela 2010b; Rice 1984). Sexual dimorphism may also include sex-specific gene silencing, which may be affected by the sex chromosomal complement and not just the presence of the heteromorphic sex chromosome (Wijchers et al. 2010). Given the extent of the consequences of sex chromosome evolution (e.g., Bachtrog et al. 2011; Valenzuela 2010b; Pipoly et al. 2015; Rice 1984; Connallon and Jakubowski 2009), the question remains as to whether sexual dimorphism and sexual selection/conflict are less pronounced in species with strict TSD that lack sex chromosomes as appears to be the case for some reptiles (Valenzuela et al. 2014).

12.7 Conclusion

Reptiles hold a crucial key to deciphering the puzzle of the diversity of sex determination, the proximate mechanism that regulate sexual development, and the ultimate forces that drive its evolution. Comprehensive studies that integrate information across these levels, about the molecular architecture of sexual development and how it evolves under the particular ecological contexts of various lineages, will shed light on the causes and consequences of the evolution of sex determination. Despite significant efforts thus far, further research is needed to inventory the full extent of this diversity as our knowledge remains fragmentary, and new analytical methods require development to allow the reconstruction of its evolutionary history.

However, the rapid pace at which new technologies and phylogenetic methods are advancing is encouraging, and they should provide a comprehensive understanding of how and why nature leaves the control of such a fundamental process as is the production of males and females vulnerable to external inputs, and when it canalizes it away from environmental whims.

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