

Evolutionary transitions between mechanisms of sex determination in vertebrates

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Sex in many organisms is a dichotomous phenotype—individuals are either male or female. The molecular pathways underlying sex determination are governed by the genetic contribution of parents to the zygote, the environment in which the zygote develops or interaction of the two, depending on the species. Systems in which multiple interacting influences or a continuously varying influence (such as temperature) determines a dichotomous outcome have at least one threshold. We show that when sex is viewed as a threshold trait, evolution in that threshold can permit novel transitions between genotypic and temperature-dependent sex determination (TSD) and remarkably, between male (XX/XY) and female (ZZ/ZW) heterogamety. Transitions are possible without substantive genotypic innovation of novel sex-determining mutations or transpositions, so that the master sex gene and sex chromosome pair can be retained in ZW–XY transitions. We also show that evolution in the threshold can explain all observed patterns in vertebrate TSD, when coupled with evolution in embryonic survivorship limits.

Keywords: sex differentiation; temperature-dependent sex determination; genetic sex determination; sex chromosome; gene–environment interaction

1. INTRODUCTION

Vertebrates show an astonishing array of mechanisms that govern sexual phenotype and so provide good models for exploring the evolution of sex-determining mechanisms. Mammals other than monotremes have male heterogamety (XX females/XY males)—maleness is usually determined by a master gene, *SRY*, on a distinct Y chromosome [1,2]. Birds have female heterogamety (ZZ males/ZW females) with distinctly differentiated sex chromosomes (except Ratites)—double dosage of the Z-borne gene *DMRT1* determines male development [3]. Fishes, amphibians and reptiles may have male or female heterogametic genetic sex determination (GSD) with or without strongly

differentiated sex chromosomes. In addition, many reptiles and some fishes have environmental sex determination (ESD), most commonly in the form of temperature-dependent sex determination (TSD) [4,5]. For some, temperature and genotype interact to determine sex [6–8]. Sex ratios of TSD reptiles exhibit three general patterns of response to temperature: males at low temperature, females at high (MF or Type 1A); females at low, males at high (FM or Type 1B); and females at low and high with males (or both sexes) at intermediate temperatures (FMF or Type II) [9].

The diversity of sex-determining mechanisms in reptiles and fishes suggests a complex evolutionary history of transitions between sex determination modes [10,11]. TSD can evolve from or to male or female heterogamety, provided that there is temperature sensitivity in the GSD mechanism and selection for different levels of that sensitivity [12,13]. Bull [4,5] showed that there may be continuous paths of selectively neutral equilibria between strict GSD and strict ESD, and between male and female heterogamety. We build upon these earlier insights by proposing a simple model for sex determination evolution, which incorporates the effect of sex gene dosage and changes in the level of thermosensitivity within a population.

We base our model upon the one or more thresholds that must exist in any system in which a continuously varying factor (such as temperature) or multiple interacting genetic factors determine a dichotomous outcome (such as sex) [14]. Such thresholds are well established in TSD reptiles [9,15] and exist also where multiple genes interact in mammalian sexual determination [16]. In our model, the sex-determining threshold is the minimum regulatory signal required to shift the balance between the competing signals directing the opposing male and female developmental programmes [17,18]. Feedback signalling loops during subsequent sex differentiation reinforce any imbalance in those competing signals to commit gonadal differentiation to one fate only [19]. Depending on the species, temperature (TSD) or the presence or dosage of a master sex-determining gene (GSD) has the initial primary influence on this balance. Genetic variation in the genes governing sex determination and the efficacy of the transmission and reception of their regulatory signalling will establish different set points for the sex-determining threshold, such that it is an evolutionarily labile trait. We show that evolution in that threshold permits novel transitions between GSD and TSD systems, between male and female heterogamety and, in the specific case of reptiles, between all known modes of TSD, without major structural innovation in the chromosomes or networks of genes that drive the regulatory processes of sex determination and differentiation.

2. THE MODEL

We start with a simple system of heterogamety in which one of the sexes is reversed at extreme incubation temperatures. We assume that sex chromosomes show little differentiation, a state common in many reptiles, amphibians and fishes [20], such that sex-reversed individuals and YY or WW genotypes are viable and fertile. YY genotypes are indeed viable in

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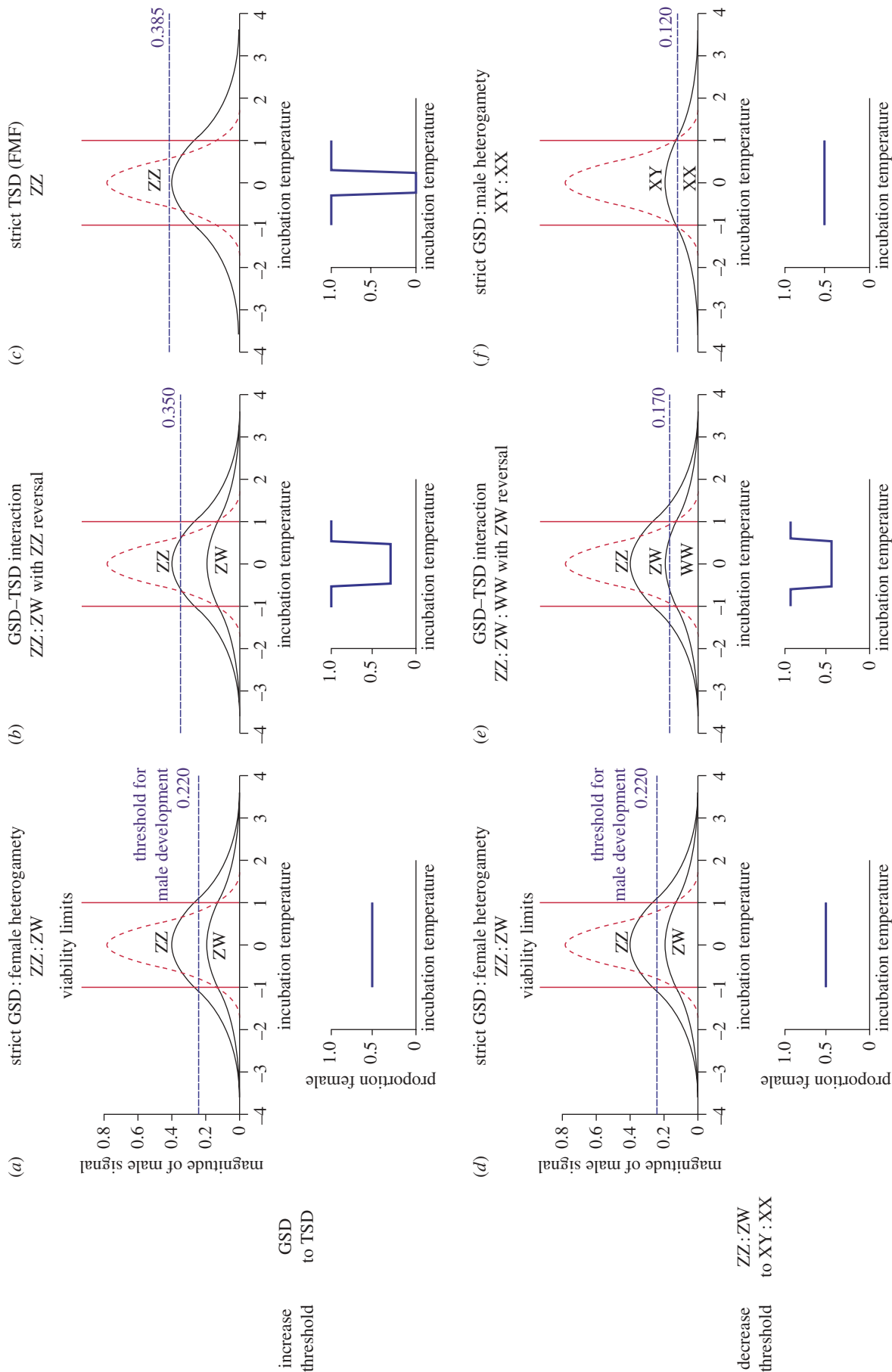
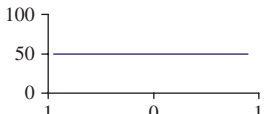
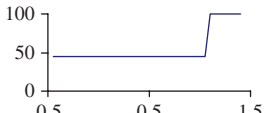
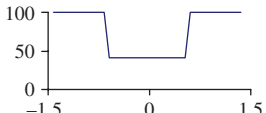
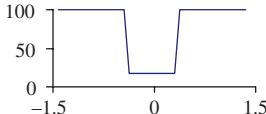
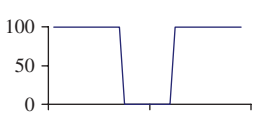
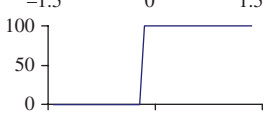
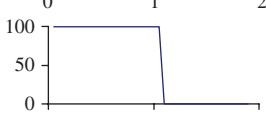
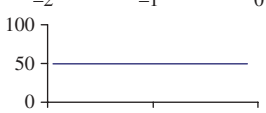


Figure 1. Transitions between sex-determining mechanisms caused by shifts in the sex-determining threshold. Black curves, magnitude of the regulatory signal for male development (arbitrarily scaled); dashed blue line, threshold value for male development; dashed red line, nest site distribution; vertical red lines, upper T_H and lower T_L thermal limits for embryo viability; solid blue line, population thermal reaction norm for sex ratio. (a–c) Effect of increasing the threshold for male development; (d–f) the effect of decreasing the threshold.

Table 1. Example model responses for different settings of the threshold, nest survival and thermal limits of embryo viability.

system of sex determination	threshold (viability limits)	genotypic equilibrium frequencies (male : female)	nest site distribution: s.d. (nest survival)	reaction norm: % females versus temperature	examples
A classic ZZ : ZW GSD	0.22 (-1.0, 1.0)	50% ZZ 50% ZW (1 : 1)	0.5102 (95%)		<i>Elaphe quadrivirgata</i> [21]; <i>Pelodiscus sinensis</i> [22]
B ZZ : ZW GSD with ZZ sex reversal	0.21 (-0.5, 1.5)	55% ZZ 45% ZW (1 : 1)	0.5102 (95%)		<i>P. vitticeps</i> [7]
C ZZ : ZW GSD with ZZ sex reversal	0.32 (-1.5, 1.5)	58% ZZ 42% ZW (1 : 1)	0.4559 (99.9%)		<i>A. muricatus</i> [23]; <i>A. impalearis</i> [24]
D ZZ : ZW GSD with ZZ sex reversal	0.37 (-1.5, 1.5)	82% ZZ 18% ZW (1 : 1)	0.4559 (99.9%)		<i>P. lesueurii</i> [15]; <i>Crocodylus porosus</i> [25]; <i>Chelydra serpentina</i> [26]
E FMF-type TSD	0.37 (-1.5, 1.5)	100% ZZ 0% ZW (41 : 59)	0.7653 (95%)		<i>Alligator mississippiensis</i> [25]
F MF-type TSD	0.265 (0, 2.0)	100% ZZ 0% ZW (42 : 58)	0.5102 (95%)		<i>Carettochelys insculpta</i> [27]; <i>Trachemys scripta</i> [28]
G FM-type TSD	0.265 (-2.0, 0)	100% ZZ 0% ZW (42 : 58)	0.5102 (95%)		<i>Sphenodon punctatus</i> [29]
H classic XX : XY GSD	0.11 (-1.0, 1.0)	50% ZW 50% WW (1 : 1)	0.5102 (95%)		<i>Chelodina longicollis</i> [20,30]

some species of amphibian and fishes. Our model is unlikely to apply to most mammals and birds because of the high degree of degeneration of the Y and W chromosomes and the probable lethality of YY and WW combinations.

We begin with a ZZ/ZW system where a double dose (ZZ) of a Z-borne male-determining gene generates an integrated male-determining regulatory signal above a threshold necessary for testes development (electronic supplementary material). When this gene is present only in a single dose (ZW), the regulatory signal is halved and falls below the threshold required for testes development. Ovarian development ensues. Sex is reversed by temperature in several species with sex chromosomes (e.g. [7,8,31,32]), so we further include temperature sensitivity in the overall regulatory signal (electronic supplementary material, figure S1). Temperature at both extremes diminishes the male-determining signal such that, at some point subject to survivorship constraints, ZZ individuals are reversed to female phenotypes. Temperature could conceivably exert its influence at any of several points in the

regulatory cascade [33] (not necessarily the master trigger itself). However, as with threshold and catastrophe models generally, the utility of our model does not rely on detailed knowledge of the many internal variables governing the overall integrated regulatory signal, upon which the external variable, temperature, exerts its influence.

Having set a particular value for the threshold for male development, and converted initial genotypes to phenotypes, we imposed random mating on the population and calculated relative frequencies of each genotype in the resultant offspring population. The process was repeated for 30 generations to reach equilibrium. This approach incorporated the action of both temperature-induced sex reversal and frequency-dependent selection on the population sex ratio. The response of interest in the calculations was change in genotype frequencies and proportions of concordant and sex-reversed individuals. A key output of the model was the population thermal reaction norm for sex ratio. The model was implemented in MICROSOFT EXCEL.

Table 2. Heterogametic transitions caused by shifts in the threshold for sex determination, with consequences for dosage ratio of the master sex-determining gene.

master sex gene	dosage of master sex gene			action of master sex gene	
	females heterogamety ZZ : ZW	intermediate system ZZ : ZW : WW YY : YX : XX	male heterogamety XY : XX	threshold for male development	threshold for female development
female-determining gene on W/X	0 : 1	0 : 1 : 2	1 : 2	downregulates male signal	upregulates female signal
male-determining gene on Z/Y	2 : 1	2 : 1 : 0	1 : 0	upregulates male signal	downregulates female signal

←----- heterogametic transition -----→

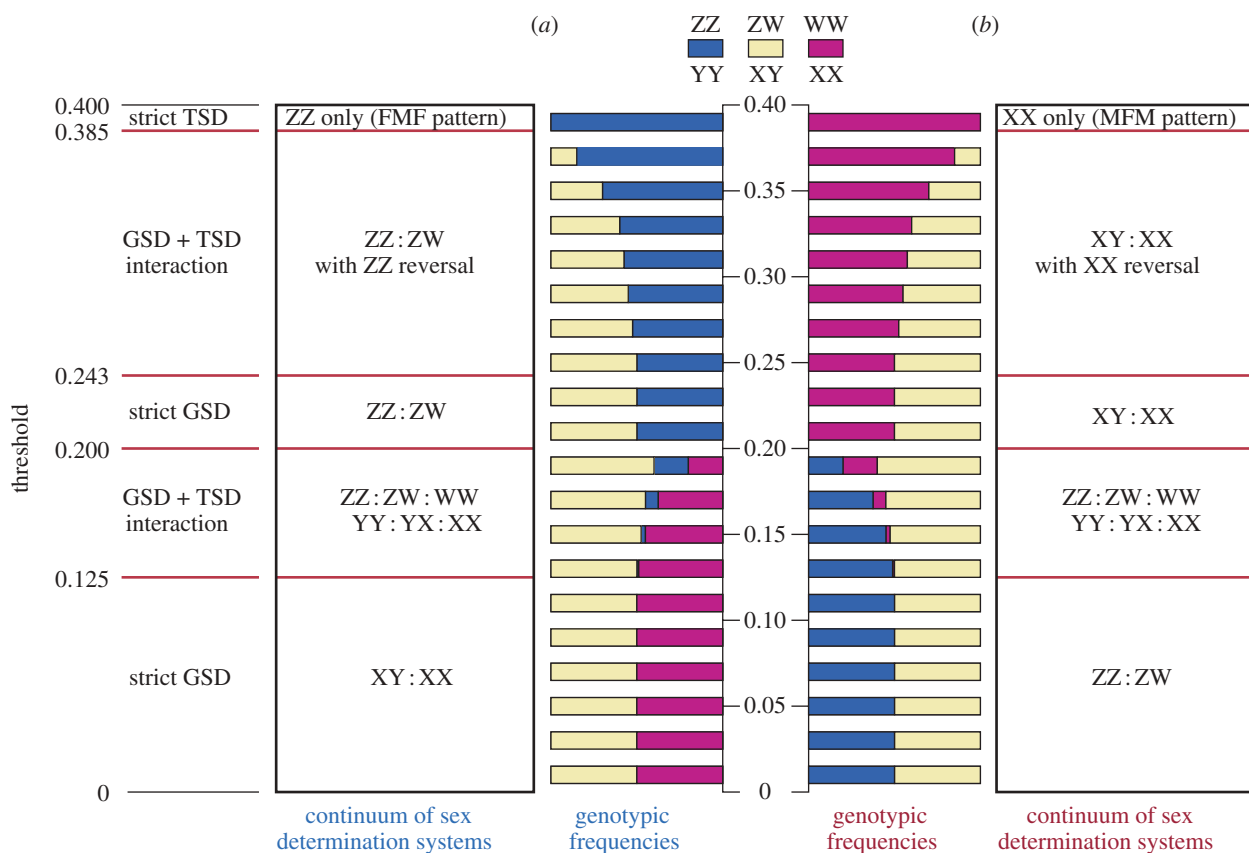


Figure 2. Evolutionary continua of sex-determining systems for populations with thermosensitivity in (a) male or (b) female differentiation. Red lines, threshold values at transition points between sex-determining systems; coloured bars, relative genotypic frequencies at equilibrium. Viability limits = $-1.0, 1.0$; nest survival = 95%; initial genotypic frequencies ZZ : ZW : WW = 1 : 1 : 1.

3. RESULTS

Setting the threshold above the initial ZZ/ZW state (figure 1a) produced a transition from GSD to TSD. As the threshold was progressively increased, the proportion of ZZ females also increased, driving down the frequency of the ZW genotype and diminishing the proportion of females produced at intermediate temperatures (figure 1b) to finally yield the typical FMF pattern of TSD. Ultimately, the ZW genotype, and thus the W chromosome, was eliminated and the sex of all embryos was determined by temperature

(figure 1c). When the thermal window of embryo survivorship was altered in addition to altering the threshold, all known patterns of temperature influence on sex ratio in reptiles (including FM and MF) were generated (table 1).

Conversely, lowering the threshold from the initial ZZ/ZW state (figure 1d) resulted in a transition from female to male heterogamety. Once the threshold was below the maximal level of the ZW regulatory signal, ZW individuals incubated at intermediate temperatures developed as males. Mating between ZW males

and ZW females produced 25 per cent WW offspring that, lacking the Z-borne gene altogether, develop as phenotypic females (figure 1e). Once the threshold was decreased beyond the point where the signal in all ZW individuals exceeded the threshold, only WW individuals were females and no ZZ individuals were produced. The population then has ZW/WW male heterogamety, which differs only semantically from an XY/XX system (figure 1f). The same pattern arises where the master sex gene is W-borne and down-regulates the male signal, thus favouring the competing female signal (table 2).

For the converse system of an XX/XY population with thermosensitivity allowing XX reversal, an analogous continuum of sex-determining modes emerges (figure 2), with the important exception that a male–female–male (MFM) pattern of GSD–TSD interaction arises, as observed in flatfish [34].

4. DISCUSSION

Our central finding is that a continuum of sex determination systems can emerge from quantitative shifts in a regulatory threshold for male (or female) development, revealing a novel pathway for heterogametic transitions via an intermediate state of GSD–TSD interaction. Threshold shifts could result from direct selection for increased (or decreased) thermosensitivity [13,35], from genetic variants in the regulatory sex network hitchhiking with linked genes undergoing positive selection, or from drift in the frequencies of those variants. In principle, directional shifts in the mean threshold value for a population could cause heterogametic transitions even in the absence of thermosensitivity in sex determination.

Previous models for heterogametic transitions typically invoke the acquisition of a novel master sex gene and sex chromosome pair [5,12,36,37]. By contrast, our model predicts that transitions are possible without substantive genotypic innovation involving novel sex-determining mutations or transpositions and de novo sex chromosomes. Furthermore, our model predicts homology between the W and X chromosomes and the Y and Z chromosomes in closely related, but opposite systems of heterogamety, as occurs in the Japanese frog *Rana rugosa* [38]. Further study of *R. rugosa* is required to see whether the same genes are involved, and indeed whether the dominant master sex-determining gene of one heterogametic system is the dosage-dependent master gene in the other, as our model predicts.

Homomorphy of sex chromosomes in reptiles, amphibians and fishes may have obscured diversity in sex-determining systems where genotype and environment interact to determine sex. Those few species examined in sufficient detail—*Pogona vitticeps*, *Bassiana duperreya* and *Menidia menidia* [6–8]—provide tantalizing indications of such diversity. In particular, our model predicts an underlying ZZ/ZW system in reptiles with FMF patterns of TSD, and provides an explanation for mixed sex ratios at intermediate temperatures. This prediction distinguishes our model from previous transitional models (e.g. [4,39]). Obvious candidates for testing these predictions include the

agamid lizard species that produce approximately 1 : 1 sex ratios at intermediate temperatures and 100 per cent females at extremes (e.g. *Amphibolurus muricatus*, *Physignathus lesueurii*, *Agama impalearis*).

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