JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12258

Novel evolutionary pathways of sex-determining mechanisms

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Keywords:

amphibians;
Charnov-Bull;
environmental sex determination;
fish;
genotypic sex determination;
reptiles;
sex-determining modes;
temperature-dependent sex determination.

Abstract

Evolutionary transitions between sex-determining mechanisms (SDMs) are an enigma. Among vertebrates, individual sex (male or female) is primarily determined by either genes (genotypic sex determination, GSD) or embryonic incubation temperature (temperature-dependent sex determination, TSD), and these mechanisms have undergone repeated evolutionary transitions. Despite this evolutionary lability, transitions from GSD (i.e. from male heterogamety, XX/XY, or female heterogamety, ZZ/ZW) to TSD are an evolutionary conundrum, as they appear to require crossing a fitness valley arising from the production of genotypes with reduced viability owing to being homogametic for degenerated sex chromosomes (YY or WW individuals). Moreover, it is unclear whether alternative (e.g. mixed) forms of sex determination can persist across evolutionary time. It has previously been suggested that transitions would be easy if temperature-dependent sex reversal (e.g. XX male or XY female) was asymmetrical, occurring only in the homogametic sex. However, only recently has a mechanistic model of sex determination emerged that may allow such asymmetrical sex reversal. We demonstrate that selection for TSD in a realistic sex-determining system can readily drive evolutionary transitions from GSD to TSD that do not require the production of YY or WW individuals. In XX/XY systems, sex reversal (female to male) occurs in a portion of the XX individuals only, leading to the loss of the Y allele (or chromosome) from the population as XX individuals mate with each other. The outcome is a population of XX individuals whose sex is determined by incubation temperature (TSD). Moreover, our model reveals a novel evolutionarily stable state representing a mixed-mechanism system that has not been revealed by previous approaches. This study solves two long-standing puzzles of the evolution of sex-determining mechanisms by illuminating the evolutionary pathways and endpoints.

Introduction

Sex in vertebrates is typically determined irreversibly during embryonic development either by chromosomal complement (genotypic sex determination, GSD; with male heterogamety, XX females/XY males or female heterogamety, ZZ males/ZW females) or by temperature experienced by the developing embryos (temperature-dependent sex determination, TSD; Bull, 1983). Few systems intermediate to GSD and TSD are known to exist in nature (Bull, 1983), suggesting that intermediate forms are evolutionarily unstable.

Whereas evolutionary transitions from TSD to GSD are considered simple and straightforward (e.g. Charlesworth, 1996; Sarre *et al.*, 2011), evolving from GSD to TSD appears to require crossing a fitness valley that is associated with temperature-dependent 'reversal' of genotypic sex. TSD is selected for when fitness depends on incubation temperature in a sex-specific fashion (Charnov & Bull, 1977), and may favour individuals that develop as the sex that benefits the most at the given temperature, regardless of genotype. However, a problem arises when considering sex-reversed

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heterogametes: when a sex-reversed XY female (or ZW male) mates with a wild-type XY male (ZW female), 25% of their offspring will be a novel genotype (YY or WW). Because GSD often involves a degenerate sex chromosome (Y or W), individuals that are homogametic for the degenerated chromosome are often nonviable or infertile (e.g. YY in XX/XY system or WW individuals in ZZ/ZW system; Bull, 1983; Charlesworth, 1996; but see Devlin & Nagahama, 2002). Even in those species with apparently homomorphic or cryptic sex chromosomes, the accumulation of male- or female-beneficial alleles on one sex chromosome owing to sexually antagonistic selection may lead to negative fitness consequences in atypical genotypes (Charlesworth & Charlesworth, 1980; Bull, 1983; Rice, 1984, 1987). This reduced viability diminishes the fitness of sex-reversed heterogametic individuals, thereby reducing the likelihood of transitioning to complete TSD and eliminating the possibility of mixed-mechanism systems of sex determination (i.e. genotypic- and temperature dependence combined).

Given these apparent challenges to evolutionary transitions, it is surprising that sex-determining mechanisms (SDMs) seem to be evolutionary labile in fish (Devlin & Nagahama, 2002; Ospina-Alvarez & Piferrer, 2008), amphibians (transitions among forms of GSD; Wallace *et al.*, 1999; Nakamura, 2009) and reptiles, particularly in lizards (Janzen & Krenz, 2004; Organ & Janes, 2008; Gamble, 2010; Sarre *et al.*, 2011). TSD has been estimated to have evolved independently from GSD at least five times in lizards, and there are at least six origins of different forms of GSD from TSD in turtles (Janzen & Krenz, 2004; Organ & Janes, 2008). Within the lizard family Agamidae, species with GSD and those with TSD co-occur in the same genus (Harlow, 2004). Furthermore, although the GSD–TSD dichotomy dominates our current empirical understanding of SDMs across wild vertebrates, the boundaries are sometimes obscured. A growing minority of GSD systems demonstrates sex reversal of individuals at extreme temperatures (e.g. three-lined skink, Shine *et al.*, 2002; Radder *et al.*, 2008; bearded dragons, Quinn *et al.*, 2007; fish, Devlin & Nagahama, 2002; amphibians, Wallace *et al.*, 1999). Similarly, for species with TSD, genetic variance is known to influence sex, revealed through significant heritability of the sex ratio at given temperatures (Bull *et al.*, 1982; Janzen, 1992; McGaugh & Janzen, 2011; Rhen *et al.*, 2011) and the apparent existence of genotypes that are female at all temperatures (based on a lack of 100% male temperatures; e.g. Harlow, 2004).

How, then, can we explain evolutionary lability and rare intermediate forms? Most theory on the evolution of temperature-dependent sex determination does not specify underlying genetics, thus avoiding the issue of sex reversal of genotypic forms (e.g. Van Dooren & Leimar, 2003, Schwanz & Proulx, 2008; Schwanz *et al.*, 2010). Empirically, temperature-induced sex reversal in GSD systems often affects the homogametic sex only – XX males in three-lined skinks and medaka (Shine *et al.*, 2002; Sato *et al.*, 2005; Radder *et al.*, 2008; but see counterexamples in fish, Wallace *et al.*, 1999; Devlin & Nagahama, 2002) and ZZ females in bearded dragons (Quinn *et al.*, 2007). A population genetics model by Bull (1981, 1983) specified such an asymmetrical scenario of sex reversal to examine the evolutionary transition from GSD to TSD. This previous model showed that, if only XX individuals are sex-reversed (to males) by temperature, potentially nonviable YY genotypes are never produced and the Y allele disappears from the population. However, this asymmetry in sex reversal is a large and critical assumption, and its mechanistic validity has remained a matter of conjecture. We suspect that misgivings over this assumption have led to the continued doubt of simple evolutionary transitions from GSD to TSD. Recently, a static, mechanistic model of sex determination (Quinn *et al.*, 2007, 2011; Fig. 1a) was proposed that suggested a mechanistic explanation for asymmetrical sex reversal. Here, we place this mechanistic view within an evolutionary model to examine evolutionary transitions among sex-determining systems. We reveal two crucial findings. Firstly, once mechanisms are specified, selection for TSD causes evolutionary transitions from XX/XY or ZZ/ZW systems to TSD in a manner similar to that assumed by Bull (1981). The specified mechanism naturally avoids the production of potentially nonviable or infertile YY or WW genotypes, thereby confirming the evolutionary pathway and overturning conventional assumptions about these evolutionary transitions. Secondly, we discover a previously unknown evolutionary endpoint that incorporates both genotypic and temperature dependence in sex determination – a mixed-mechanism stable state that has previously been assumed impossible and that was not apparent with previous analytical approaches (Bull, 1981, 1983).

Materials and methods

Model overview

We developed an agent-based simulation model to examine the evolutionary transitions from an established GSD system to TSD, building on a recent mechanistic model by Quinn *et al.* (2011). Our approach is distinct from a traditional population genetics view in that it explicitly models reaction norms of sexual phenotype for different genotypes. We considered a sex-determining locus located on the sex chromosomes, with two alleles: one allele with a high level of expression (A, with expression A_{signal}) and the other allele with a low level of expression (a, with expression a_{signal}). Genotypic levels of signal expression (S_g) are the average of the two allelic values. Signal expression is influenced by embryonic incubation temperature

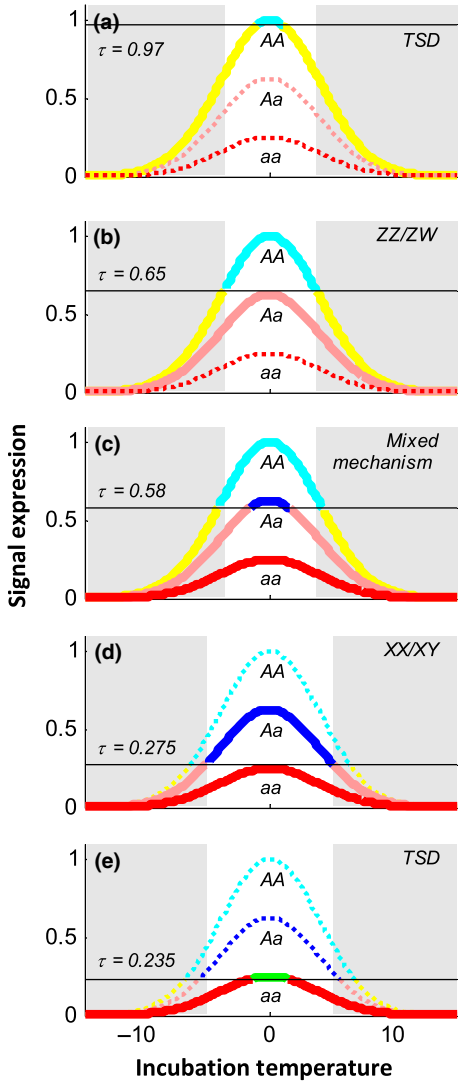

Fig. 1 Genotypic signal expression and its relation to sex-determining mechanisms. Expression of a male signal depends on genotype (*AA*, *Aa* or *aa*) at a sex-determining locus as well as incubation temperature (coloured thick solid lines indicate genotypes present in the population, whereas dotted lines indicate genotypes not present). Incubation temperatures occur within with unshaded portion of each graph in 99% of individuals [variance in incubation temperatures is 1.37 in (a–c) and 1.89 in (d) and (e)]. Development as male occurs when the male signal exceeds a threshold level, τ. Simulations were initiated as either ZZ/ZW ((b); *AA* individuals are males (ZZ), *Aa* individuals are females (ZW), *aa* genotype is not present) or XX/XY [(d); *AA* genotype is not present, *Aa* individuals are males (XY), *aa* individuals are females (XX)]. Under selection for TSD, the threshold, τ, evolved upwards or downwards to either temperature-dependent sex determination [TSD) with only *AA* (ZZ, (a)] or *aa* [XX, (f)] genotypes or to a mixed-mechanism system (c) with all genotypes present and the sex of the heterozygote strongly dependent on temperature. Colours reflect genotype-by-sex combinations: *AA* males (cyan), *AA* females (yellow), *Aa* males (blue), *Aa* females (pink), *aa* females (red) and *aa* males (green).

such that there is a temperature of peak expression and expression declines for all genotypes away from this peak, as given by a normal curve [$S_g(T)$; with mean μ_{signal} and standard deviation σ_{signa1}]. Thus, signal expression is a reaction norm as a function of temperature, and the height of the reaction norms (peak signal expression) differs among genotypes at the sex-determining locus (Fig. 1). These reaction norm curves differ from traditional population genetics approaches in that they plot two variables orthogonal to each other (signal expression and temperature), rather than representing frequency distributions of one of these variables as might be assumed by the use of a normal curve. Development into a male or female during gonadogenesis depends on whether the genotypic expression level exceeds a threshold, τ [male if $S_g(T) > \tau$], which is determined by an unlinked, autosomal locus. The temperatures at which genotypic expression crosses the threshold level are the pivotal temperatures (Fig. 1).

Female heterogamety, male heterogamety, TSD and the *A*/*a* genotypes

This general genetic set-up can represent male heterogamety, female heterogamety and temperature-dependent sex determination, depending on the genotypes present and the level of the threshold (Table 1; Fig. 1). This ability to encompass all of these forms of sex determination makes the model more flexible and all-encompassing than traditional population genetics approaches that do not specify reaction norms. In general, female heterogamety (ZZ/ZW) is represented by genotypic systems where *AA* individuals are above the threshold of maleness and thus develop as males, and *Aa* individuals are below the threshold and develop as females (Fig. 1b). This is consistent with the ZZ-dosage effect likely operating in birds (Smith *et al.*, 2009). Thus, in our simulations, a ZZ/ZW system consists of all *AA* and *Aa* genotypes, and a mean τ just above the *Aa* peak expression level (Fig. 1b). Male heterogamety (XX/XY) is represented by genotypic systems with a lower threshold, where *Aa* individuals are male and *aa*

Table 1 Genotypic relationships and simulated signal peaks. The simulation was specified with three genotypes, based on alleles *A* and *a*. The genotypes have different levels of peak signal expression. These genotypes can represent sex chromosomes in both ZZ/ZW and XX/XY systems, depending on the threshold of signal required to develop as a male, τ.

General genotype	Female heterogamety	Male heterogamety	Peak signal expression*
aa	WW	XX	0.25
Aa	ZW	XY	0.625
AA	ZZ	YY	1

*Based on parameterization of the simulations in this article.

individuals are female (Fig. 1d), which is consistent with the Y-specific gene effect in operation as observed in most mammals (Koopman *et al.*, 1990). In our simulations, a XX/XY system consists of *Aa* and *aa* genotypes, and a mean τ just above the *aa* peak expression level (Fig. 1d). TSD is characterized by systems where a single genotype is present (*AA* or *aa*), and individuals of this genotype are above or below the threshold, τ, depending on their incubation temperature (Figs 1a, e).

Temperature and survival

Incubation temperature is chosen from a normally distributed probability distribution of the mother's nesting behaviour (with mean (μ_{nesting}) and standard deviation (σ_{nesting})). Embryonic relative survival probability is influenced by temperature according to a normal distribution (Fig. 2a; with mean μ_{survival} and standard deviation σ_{survival}). Because survival probability is relative to other offspring (see model details below), the exact value of the survival probability is arbitrary. To examine the impact of reduced viability of YY or WW individuals on the simulation results, the relative survival of WW (*aa* in ZW system) and YY *(AA* in XY system) individuals was parameterized as a proportion of the relative survival of other genotypes (ρ; Fig. 2a). XX males and ZZ females were assumed to have equal fitness to XY males and ZW females, respectively.

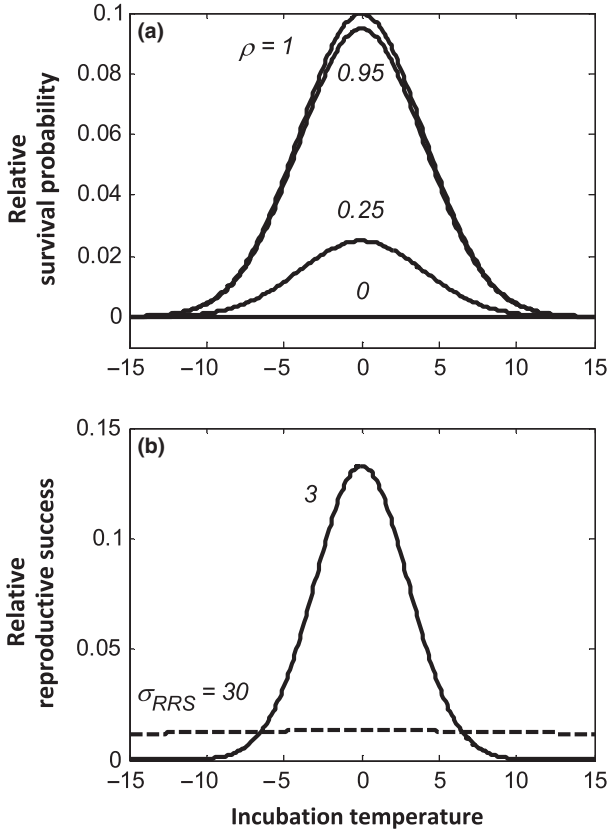

Fig. 2 (a) Relative embryonic survival probability depends on incubation temperature (values on *y*-axis are arbitrary), with WW and YY individuals having relative proportional survival that may be equal to (ρ = 1) or less than (ρ = 0.95, 0.25 or 0) the other genotypes (ZZ and ZW, or XX and XY). (b) To select for temperature-dependent sex determination, relative reproductive success (RRS) of males depends strongly on incubation temperature ($\sigma_{\text{RRS},\male}$ = 3, solid line), whereas that for females has little dependence on temperature ($\sigma_{\text{RRS},\female}$ = 30, dashed line).

Selection for TSD

To vary whether there is selection for TSD, relative reproductive success (RRS, the likelihood of contributing to the next generation of offspring compared with other members of the same sex) was specified separately for males ($\mu_{\text{RRS},\male}$; $\sigma_{\text{RRS},\male}$) and females ($\mu_{\text{RRS},\female}$; $\sigma_{\text{RRS},\female}$) as a function of original incubation temperature using a normal distribution (Fig. 2b). Similar to survival, the exact values of relative reproductive success are unimportant; only the value relative to competitors is important. We can select for a common pattern of TSD, whereby females are produced at extreme temperatures and males at intermediate temperatures (female–male–female; FMF) by specifying that relative reproductive success depends on temperature more strongly in males than in females ($\sigma_{\text{RRS},\female} > \sigma_{\text{RRS},\male}$; Charnov & Bull, 1977; Fig 2b). Selection of this manner could arise if extreme temperatures lead to minor developmental instability that more greatly impacts the reproductive success of males than females due to greater intrasexual competition in the former.

Example

To illustrate how the model works, imagine an XY (*Aa*) individual with a threshold much lower than its male signal peak (0.275 in our parameterizations, Fig. 1d). If this individual develops at an incubation temperature value of 1, it will develop as a male and have a high relative survival probability and RRS (competing against other males). If that same genotype were to develop at a temperature of 3, he would have a relatively low survival probability and a very low RRS competing against other males. In contrast, an XX (*aa*) individual with a threshold just below its peak (0.22; Fig. 1e) will also develop as a male at a temperature of 1 and enjoy high relative survival and RRS, but will develop as a female at a temperature of 3, having low relative survival, but similar RRS compared with other females. The exact survival probability and reproductive success of each individual depend on the other genotypes in the population against which individuals compete for survival to adulthood and contribution of offspring to the next generation. Thus, under selection for TSD, genotypic combinations are favoured that

capitalize on high RRS of males at intermediate temperatures by developing as a male at these temperatures while avoiding low RRS of males at extreme temperatures by developing as a female at extreme temperatures.

Model details and evolutionary scenarios

A population consisting of 1000 adults was initiated with GSD. We varied the starting genetic system as ZZ/ZW (Fig. 1b; starting τ specified in Table 2; *AA* and *Aa* genotypes assigned randomly at model initiation) or XX/XY (Fig. 1d; starting τ specified in Table 2; *Aa* and *aa* genotypes assigned randomly at model initiation). The standard deviation of the nesting temperature distribution differed according to whether the starting system was ZZ/ZW (SD = 1.37) or XX/XY (SD = 1.89) to standardize the probability of producing sex-reversed individuals at extreme temperatures at model initiation. For example, at our standard starting values of τ (ZZ/ZW: 0.65; XX/XY: 0.275), there was approximately 1% chance of a clutch temperature that would produce females if the genotype were ZZ or XY; thus, approximately 0.5% of individuals were sex-reversed. We specified this minor amount of sex reversal at model initiation so that mutational space around the standard starting values of τ could lead to sex reversal in either way. That is, if the threshold mutates downward a small amount to fall below the peak of the lower genotypic curve, this has a very strong effect on the number of individuals that are sex-reversed, driven by the nature of a normal distribution of nest temperatures. In contrast, upward mutations in the threshold lead to a gradual increase in the number of sex-reversed individuals (Fig. 3).

To simulate reproduction, males were sampled from the male mating pool based on a male's RRS (relative reproductive success) and assigned to each adult female. A female was assigned a nesting temperature based on the nesting (incubation) temperature distribution. 10 000 embryos were then sampled from the mated pairs based on each female's RRS.

Alleles of the diploid locus of major sex-determining effects were inherited by embryos in a Mendelian fashion. For computational tractability, τ was considered to be a haploid, quantitative trait and was inherited randomly from either the mother or the father (assuming haploid or diploid here is unlikely to alter the evolutionary response in traits, Kokko, 2007). Mutation occurred in 2% of embryos. For a range of parameters (see above and Table 2), we examined evolution in the threshold for male development, τ, by allowing this parameter to mutate during the simulation. In mutants, a new value of τ was chosen from a normal distribution with a mean of the inherited value and a parameterized mutational standard deviation ($\sigma_{\text{mutation},\tau} = 0.02$). Subsequent to mutation, sex and relative survival probability for each embryo were determined based on incubation temperature, genotype at the locus of major effect, threshold to become male and relative survival of YY or WW embryos. Following reproduction, all adults died (nonoverlapping generations) and 1000 new adults were sampled from the embryo pool based on relative survival probability. The life cycle was iterated 10 000 times.

For each SDM, we specified selection for TSD ($\sigma_{\text{RRS},♀} > \sigma_{\text{RRS},♂}$) and examined four levels of relative survival of WW or YY embryos (Table 2) with starting $\tau = 0.65$ (ZZ/ZW) or $\tau = 0.275$ (XX/XY). We replicated each of these scenarios 10 times. In addition, for both ZZ/ZW and XX/XY systems and the extremes of relative survival of WW or YY embryos ([0,1]), we examined the effects of starting conditions by setting the starting value of τ to five additional, equally spaced values

Table 2 Definition and values of parameters in the model.

Parameter	Definition	Values
Evolving		
τ	Inherited threshold signal value for developing as a male; values at initiation of simulation presented	ZZ/ZW: 0.65,0.715,0.78, 0.845,0.91,0.975 XX/XY: 0.275,0.34,0.405, 0.47, 0.535,0.6
Fixed		
A_{signal}	Scaled signal strength of the *A* allele	1
a_{signal}	Scaled signal strength of the *a* allele	0.25
μ_{signal}; σ_{signal}	Mean and standard deviation of signal strength as a normal curve of temperature	0; 4
μ_{survival}; σ_{survival}	Mean and standard deviation of relative embryonic survival as a normal curve of temperature	0; 4
μ_{nesting}; σ_{nesting}	Mean and standard deviation of nesting (incubation) temperature as a normal pdf* of temperature	0; 1.37 (ZZ/ZW) 0; 1.89 (XX/XY)
$\mu_{\text{RRS},♂}$; $\sigma_{\text{RRS},♂}$	Mean and standard deviation of relative male reproductive success as a normal curve of temperature	0; 3
$\mu_{\text{RRS},♀}$; $\sigma_{\text{RRS},♀}$	Mean and standard deviation of relative female reproductive success as a normal curve of temperature	0; 30
ρ	Proportional survival of YY or WW embryos compared with other genotypes	0, 0.25, 0.95, 1

*Probability density function.

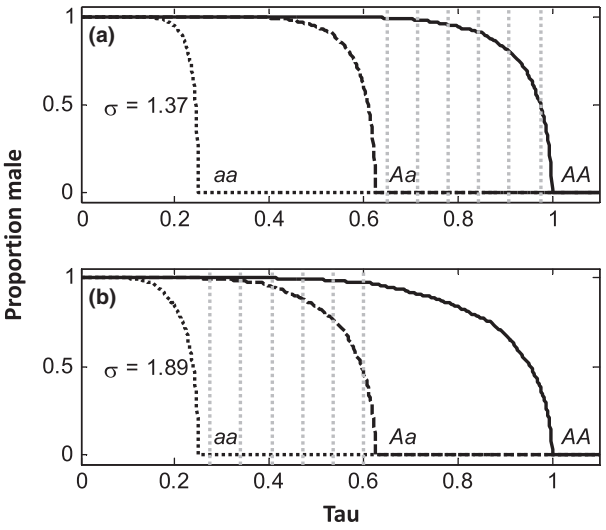

Fig. 3 The proportion of individuals developing as male (versus female) for each genotype, *aa* (dotted black line), *Aa* (dashed black line) and *AA* (solid black line), depending on the value of the threshold τ. The relationships differ according to the standard deviation in incubation temperature, shown here for the values used in simulation initiated as ZZ/ZW (a) and XX/XY (b). The vertical, dotted grey lines represent the starting values of τ examined in replicate simulations for the respective initial GSD system.

(Table 2; replicated five times at each starting τ). Finally, because we observed the evolution of two alternative evolutionary stable states (pure TSD and mixed mechanism, see results), we compared the relative stability of these two states by examining (i) reciprocal invasion ability and (ii) individual fitness in the two types of populations (see Appendix 1).

The model was implemented in MATLAB r2011a (Mathworks, Natick, MA, USA). A tested version of the model in R is provided as Data S1–S12. The provided model allows evolutionary biologists to study the evolutionary dynamics of TSD–GSD systems for their specific cases and will serve as a valuable tool to test further and more specific hypothesis.

Results

Evolution of TSD

Our model revealed that selection for TSD caused evolutionary transitions from GSD to TSD without the production of nonviable YY or WW individuals. Under complete nonviability of WW or YY individuals, the threshold (τ) consistently evolved upwards (ZZ/ZW, 10/10 replicates; Table 3; Fig. 4) or downwards (XX/XY, 10/10 replicates; Table 3; Fig. 4), leading to sex reversal of the homogametic sex only (ZZ or XX; Fig. 5a,b). As the frequency of these sex-reversed individuals increased, the frequency of heterogametic individuals decreased (ZW females and XY males; Fig. 5a,b), presumably due to increasingly frequent matings between homogametic males and females. When the heterogamete had gone extinct, the evolutionary outcome was a pattern consistent with the FMF (female–male–female) pattern of TSD with a single genotype at the major sex-determining locus (Fig. 5d,e).

Table 3 Evolutionary outcome of 10 replicated simulations initiated as genotypic sex-determination (GSD: ZZ/ZW or XX/XY) and subject to selection for temperature-dependent sex determination (TSD). Three outcomes were observed after 10 000 iterations: maintenance of the initial GSD system, evolution of a TSD system with a single genotype at the major sex-determining locus, or evolution of a mixed-mechanism (MM) system, where both genotype and temperature-influenced sex determination. Chi-squared tests were used to assess whether the outcomes differed from an expectation of random assignment into each of the three outcome categories.

Relative survival of WW or YY	ZZ/ZW (starting τ = 0.65) Outcome: GSD/TSD/MM	XX/XY (starting τ = 0.275) Outcome: GSD/TSD/MM
0	0/10/0*	0/10/0*
0.25	0/10/0*	0/10/0*
0.95	3/7/0†	0/10/0*
1	0/0/10*	0/10/0*

*$\chi^2_2 = 20$, $P < 0.0001$.
†$\chi^2_2 = 7.4$, $P = 0.02$.

Similar results were seen when viability of YY and WW embryos was higher than zero (Table 3). Even with completely viable YY individuals, we consistently observed the evolution of TSD through sex reversal of XX individuals only (reduced τ and X fixation; Table 3; Fig. 4). When considering a system that starts with ZZ/ZW, the results were qualitatively the same even under minor viability costs to WW embryos (95% survival compared with other genotypes). Here, the threshold was still significantly more likely to evolve upwards to a system of TSD, but remained near the starting value in a ZZ/ZW system in three replicates (Table 3; Fig. 4). In contrast, when WW embryos were completely viable, a different evolutionary outcome was consistently observed. Here, τ evolved downward in all cases (10 of 10) to an apparently stable value (approximately 0.58) that produced a mixed-mechanism system described by the existence of WW females, ZZ males and ZW individuals of both sexes (Table 3; Figs 4 and 5c,f).

Alternative states

If selecting for TSD leads to the evolution of two alternative states for the population (pure TSD and mixed mechanism), are the two states equal or is one more stable than the other? The starting value of τ had little influence on the evolutionary outcome when YY or

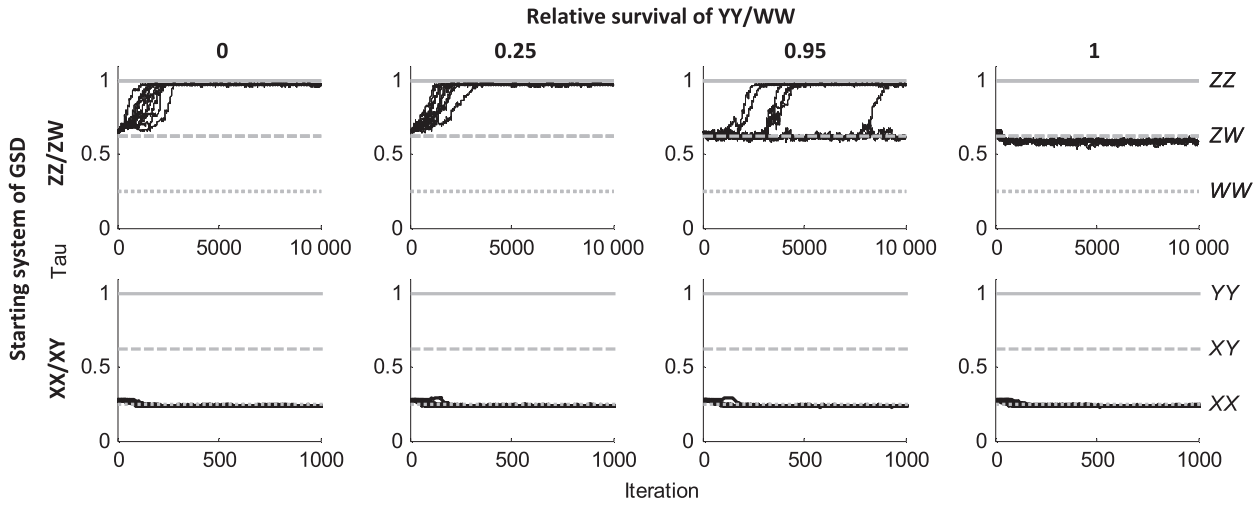

Fig. 4 Trajectory of mean τ over simulated evolutionary time for populations subjected to selection for temperature-dependent sex determination. Results are shown for populations established with a GSD system of ZZ/ZW (top row; starting $\tau = 0.65$; $\sigma_{nesting} = 1.37$) or XX/XY (bottom row; starting $\tau = 0.65$; $\sigma_{nesting} = 1.89$; only first 1000 iterations shown) and for four different values of relative survival of YY or WW individuals (columns). Black lines present the mean τ in each of ten replicates per panel. The grey, horizontal lines represent the signal peaks for the three genotypes (ZZ/ZW/WW or XX/XY/YY). All other parameter values can be found in Table 1.

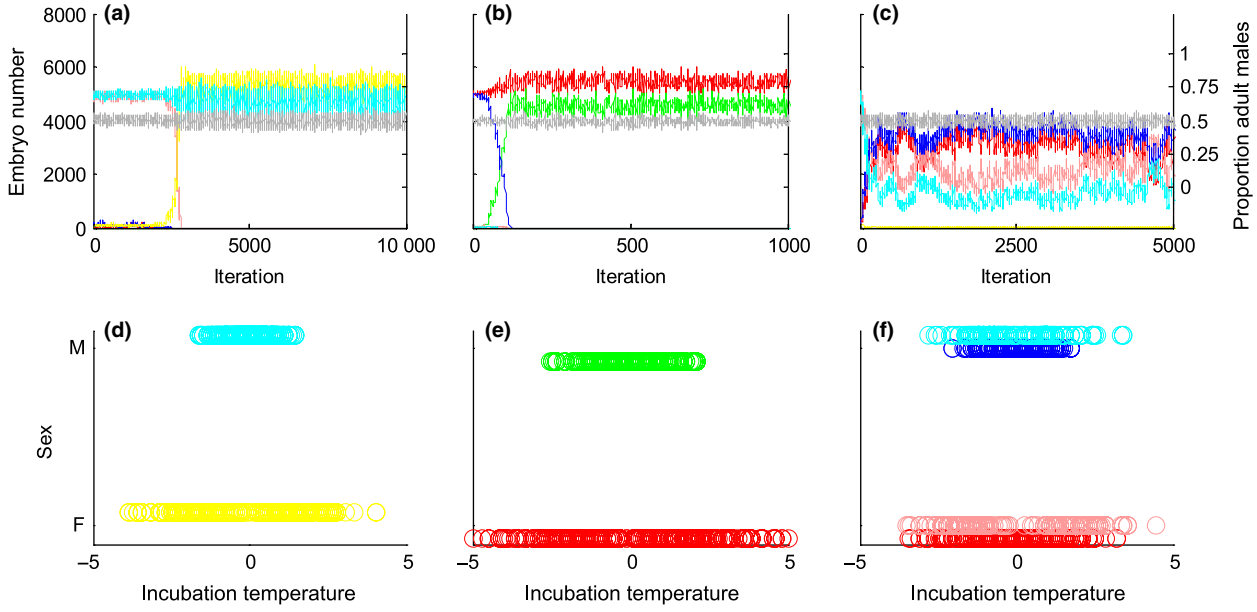

Fig. 5 Exemplary results for simulations, showing numbers of embryos produced of each genotype × sex combination over time (top row, coloured lines), adult sex ratios over time (top row, grey lines) and adult individuals by incubation temperature, genotype and sex at the end of the simulation (bottom row). Starting systems include ZZ/ZW with complete nonviability of WW (a & d), XX/XY with complete nonviability of YY (b & e) and ZZ/ZW with complete viability of WW (c & f). All other parameter values as in Fig. 4 and Table 2. In (a), ZZ males (cyan) persist and ZW males (blue) are rare, whereas ZW females (pink) disappear concomitant with the rise of ZZ females (yellow). In (b), XY males (blue) disappear at the left axis concomitant with the rise of XX males (green); XX females (red) remain at constant levels. In (c), ZZ males (cyan), ZW females (pink), ZW males (blue) and WW females (red) fluctuate in the population. Panels (d & e) show a population with TSD, whereas panel (f) shows a population with mixed mechanisms (colours same as top row). Note different *x*-axis ranges across top panels.

WW individuals were nonviable – τ moved towards sex-reversing only the homogametic sex and leading to TSD in 35/35 cases with ZZ/ZW and 32/35 cases with XX/XY (Fig. 6 circles and squares; 3/35 XX/XY replicates retained values of τ across 10 000 iterations that produced approximately 0.5% XY females). In contrast,

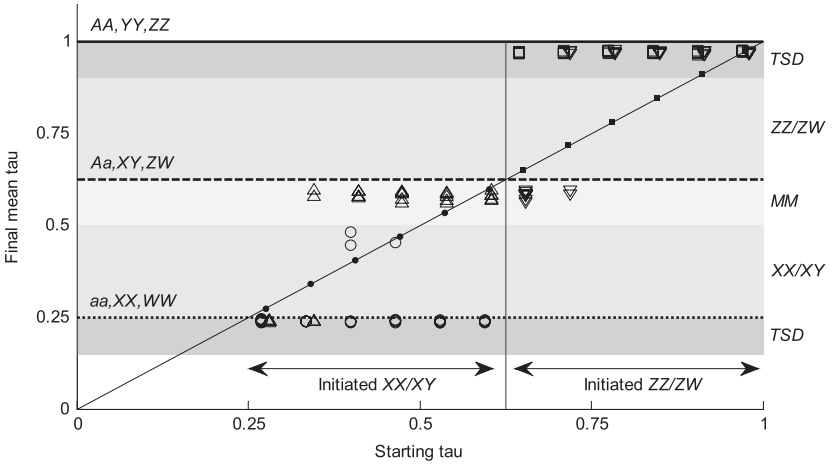

Fig. 6 The influence of starting value of the mean threshold (τ) of male signal required to become male on the evolutionary outcome of τ under selection for temperature-dependent sex determination. The solid, diagonal 1:1 line shows the 12 starting values of τ examined: six values for a system initiated with a XX/XY system (population established with *aa* and *Aa* genotypes; solid circles), and 6 values for a system initiated with a ZZ/ZW system (population established with *AA* and *Aa* genotypes; solid squares). The vertical dashed line at 'Starting τ' = 0.625 indicates the separation between the two initial systems. Each open symbol shows the final mean τ in a population of 1000 individuals after 10 000 time steps for a single replicate simulation. The grey-shade background indicates whether values of τ are typically associated with systems of TSD (with only one genotype, XX or ZZ), GSD (XX/XY or ZZ/ZW) or mixed mechanism (MM, with all three genotypes, XX/XY/YY or ZZ/ZW/WW, and temperature dependence in sex determination for the heterogametic genotype); boundaries are approximate. The bold horizontal lines show the value of the different signal peaks: *AA* (solid), *Aa* (dashed) and *aa* (dotted). Open circles are XX/XY systems where YY individuals have no survival; here, τ evolves downwards in almost all cases to a TSD system. Open squares are ZZ/ZW systems where WW individuals have no survival; here, τ evolves upwards in all cases to a TSD system. When YY (XX/XY systems; open upward triangles) or WW (ZZ/ZW systems; open downward triangles) individuals have the same survival as other genotypes, the final threshold value and representative sex-determination system (TSD or Mixed Mechanism) depend on the starting τ and the initial GSD system. Starting values of τ were replicated 10 (0.275 and 0.65, as in Fig. 4) or 5 (remainder) times for each of two survival cost scenarios (data points slightly offset on the *x*-axis to allow visualization).

the starting value of τ influenced which evolutionary state evolved when WW or YY individuals were completely viable (Fig. 6, triangles; logistic regression for log odds of evolving upwards *vs* downwards as a function of starting tau: XX/XY, $\chi^2_1 = 39.45$, $P < 0.0001$, $N = 35$; ZZ/ZW, $\chi^2_1 = 38.27$, $P < 0.0001$, $N = 35$). In both XX/XY and ZZ/ZW initial genetics, replicates with the highest three values of starting τ, which were closer to the upper equilibrium, evolved upwards to the upper equilibrium in all cases (15/15 for each of XX/XY and ZZ/ZW). Similarly, replicates with the single lowest starting τ, which was near the lower equilibrium, evolved downwards in all cases (described above). Thus, mutational proximity to the two alternative equilibria was important for determining which equilibrium the system reached. Interestingly, however, the outcomes of intermediate values of starting τ suggested that τ evolved upwards more easily than downward in both ZZ/ZW and XX/XY systems, regardless of whether the outcome was a mixed-mechanism system or TSD. Scenarios with the second lowest starting τ, which was closer in mutational distance to the lower equilibrium, evolved upwards roughly half of the time (2/5 in XX/XY and 3/5 in ZZ/ZW). Replicates with the starting τ that was roughly equidistant between the two alternative equilibria evolved to the upper equilibrium in all cases (5/5 for each of XX/XY and ZZ/ZW). If we assume a null expectation of evolving upwards or downwards randomly for the three 'intermediate' starting tau (XX/XY: 0.34, 0.405, 0.47; ZZ/ZW: 0.715, 0.78, 0.845), the upward evolution of tau occurred much more often than expected for both XX/XY ($\chi^2_1 = 10.2$, $P = 0.001$, $N = 15$) and ZZ/ZW systems ($\chi^2_1 = 11.53$, $P = 0.0007$, $N = 15$). In XX/XY, evolving to the upper equilibrium produced the mixed-mechanism system (as described in the initial results), whereas, in ZZ/ZW, evolving upwards produced TSD with the Z allele fixed.

Further examination indicates that the pure TSD form represents a more stable state than the mixed-mechanism system for the population even when YY/WW individuals are completely viable. In invasion analyses (Appendix 1), TSD mutants invaded the mixed-mechanism populations more often than expected by chance when introduced at high frequency (30% mutants invaded in 5/10 simulations, $\chi^2_1 = 1.9$, $P = 0.17$, $N = 10$; 50% mutants invaded in 8/10 simulations, $\chi^2_1 = 3.6$, $P = 0.06$, $N = 10$), although not when introduced at low frequency (10% mutants invaded with neutral expectation in 1/10 simulations, $\chi^2_1 = 0$, $P = 1$, $N = 10$). In contrast, mixed-mechanism individuals

never invaded a pure TSD population when introduced at 10, 30 or 50% of individuals in the population (each replicated 10 times), which is less than expected by chance for the higher frequencies (10%: $\chi^2_1 = 1.11$, $P = 0.29$, $N = 10$; 30%: $\chi^2_1 = 4.29$, $P = 0.04$, $N = 10$; 50%: $\chi^2_1 = 10.0$, $P = 0.002$, $N = 10$). Thus, the TSD form was better at invading a MM population than the mixed-mechanism form was at invading a pure TSD population, at least when invading at high frequency (10%: $\chi^2_1 = 1.05$, $P = 0.30$, $N = 20$; 30%: $\chi^2_1 = 6.67$, $P = 0.01$, $N = 20$; 50%: $\chi^2_1 = 13.3$, $P = 0.0003$, $N = 20$). These results suggest that the mixed form is stable from invasion of a rare TSD mutant, but that it has lower fitness when competing against a common TSD form even when YY/WW individuals are completely viable.

Examining individual fitness at the end of ZZ/ZW simulations (Appendix 1; Fig. 7) revealed that the maintenance of the mixed-mechanism system (i.e. $\tau = 0.6$) is driven by high fitness in ZW individuals (Fig. 7b,d), which are male or female depending on incubation temperature. For individuals with the threshold, τ, at the mean population value near top of the ZW peak (approximately 0.6), the fitness expected when developing as a male (Fig. 7b, blue triangles) was very high because developing as a male at only a narrow range of intermediate temperatures maximizes average male relative reproductive success and survival. For similar but less extreme reasons, fitness expected when developing as a female (pink triangles) was higher than for individuals with lower values of τ. This

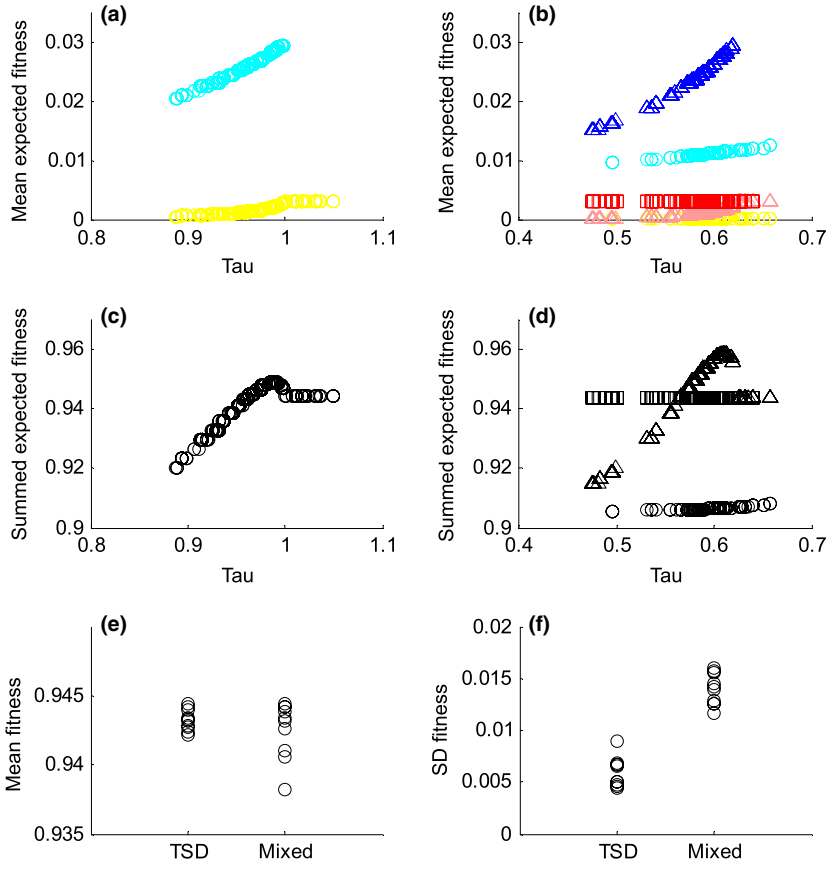

Fig. 7 Fitness of 1000 simulated individuals (10 000th iteration) in populations with pure TSD (left panels) and mixed-mechanism systems (right panels). In (a) and (b), mean expected fitness as a male or a female across sex-relevant temperatures (Appendix A) is shown for each genotype as a function of the threshold value, tau (τ), whereas (c) and (d) depict the expected fitness summed across both sexes and all incubation temperatures. In a pure TSD system (all individuals ZZ), mean fitness as a male (cyan) or a female (yellow) increases as tau increases, until tau lies above the ZZ peak (peak = 1) and fitness is only gained through female function (a). (c) Summed expected fitness in ZZ (circles) is maximized when tau is slightly < 1 (c). In a mixed system (b), mean fitness as a ZW male (blue triangles) or female (pink triangles) increases as tau increases; WW genotypes are always female (red squares) across the range of observed tau values, and there is no fitness as a male (green squares not depicted); ZZ genotypes are typically male (cyan circles), with nonzero fitness through developing as a female (yellow circles) at extreme temperatures. Fitness summed across both sexes in a MM system (d) shows that ZW individuals (triangles) with a threshold near 0.6 have high fitness compared with ZZ (circles) and WW (squares) individuals. (e) Mean and (f) standard deviation of the summed expected fitness across individuals within ten populations demonstrating pure TSD and MM.

effect of τ on ZW fitness in the MM system mirrors the same effect seen in the pure TSD system (Fig. 7a). In the mixed-mechanism system, WW individuals develop as a female across all incubation temperatures (Fig. 7b, red squares), regardless of their observed threshold of maleness, so average fitness expected as a female is equal across WW individuals with different values of τ. For ZZ individuals, individuals typically develop as a male (Fig. 7b, cyan circles) across a wide range of temperatures, so average fitness as a male is diminished compared with ZW individuals due to often being a male with reduced survival and relative reproductive success. Here, τ has a modest influence on the tendency to develop as a female (Fig. 7b, yellow circles) at extreme temperatures, which provides a nonzero average fitness as a female. Average fitness of any genotype as a female is lower than as a male largely because of the reduced survival in the extreme temperatures at which females develop.

When fitness gained through male and female function is summed across all incubation temperatures (Appendix 1, Eqn. 1), the ZW genotypes have very high expected fitness when their τ is near the final mean value (Fig. 7d, triangles). Like individuals in a pure TSD system, there is an optimal τ where development as a male at intermediate temperatures capitalizes on the very high relative reproductive success of males at these temperatures and development as a female at extreme temperatures avoids very low relative reproductive success of extreme-temperature males. ZZ individuals (Fig. 7d, circles) have low expected fitness due to the fact that, at extreme temperatures, they still develop as males and experience extremely low relative reproductive success. WW individuals (Fig. 7d, squares) have lower fitness than most ZW individuals because they are female at every temperature, thus do not benefit from high reproductive success at intermediate temperatures that they could gain if they were males. The maintenance of this value of τ is further ensured by the numerical dominance of ZW genotypes.

In contrast, in a pure TSD population, most ZZ individuals express TSD and gain the fitness advantage of linking sex to temperature (Fig. 7b). The consequences for fitness distributions within pure TSD and MM populations are clear. Overall, the mean of the summed expected fitness in each population did not differ between the ten pure TSD populations (overall mean = 0.9433) and the ten mixed-mechanism populations (overall mean = 0.9426; $F_{1,20} = 1.07$, $P = 0.31$; Fig. 7e). However, the standard deviation of individual fitness within each population was higher in the mixed-mechanism populations ($F_{1,20} = 145.25$, $P < 0.0001$; Fig. 7f). This implies that the greater stability and invasion of the pure TSD form are driven by lower within-population variance in individual fitness rather than higher mean fitness compared with the mixed-mechanism population. Because the stability of the threshold in the mixed-mechanism system depends on numerical dominance of ZW genotypes, high-frequency invasion of a pure TSD strain (e.g. all ZZ with $\tau = 0.98$) is required to overwhelm the fitness advantage of being ZW in a population where competitors are ZZ and WW with similar threshold values.

Discussion

Employing a mechanistic approach to explore the evolution of sex-determining mechanisms has revealed two primary findings. First, we demonstrate clearly that evolutionary transitions from genotypic sex determination (GSD) to temperature-dependent sex determination (TSD: with no sex chromosomes) can occur rapidly and readily without crossing a fitness valley, as first described by Bull (1981, 1983).Second, we find a novel evolutionary endpoint to selection for TSD that can best be conceptualized as a mixture of GSD and TSD.

The commonly assumed complication to transitions between SDMs – the production of viability-compromised offspring (YY or WW) by sex-reversed heterogametes (female XY or male ZW) – is completely circumvented by the evolutionary path observed in our simulations. Moving from ZZ/ZW to TSD involves an increase in the threshold (τ). This leads initially to sex reversal of ZZ embryos only (to female) at extreme temperatures, followed by the loss of the W allele and an evolutionary outcome where all individuals are ZZ and sex is determined largely according to incubation temperature. Similarly, transitioning from XX/XY to TSD involves a lowering of the threshold, such that only XX embryos are sex-reversed (to males) at intermediate temperatures, the Y allele is lost, and the outcome is all XX individuals, with sex reflecting incubation temperature. Sex reversal of only the homogametic sex was proposed by Bull (1981, 1983) as a pathway through a continuum of selectively neutral equilibria between GSD and TSD along which populations may persist. The prediction rested on the assumption that asymmetrical sex reversal is mechanistically plausible. We show here that including a mechanistic underpinning (Quinn *et al.*, 2011) validates this assumption and confirms the prediction of a simple evolutionary transition. Moreover, we demonstrate that the system moves rapidly through intermediate states rather than persisting in them. Thus, if the mechanistic model of sex determination employed here correctly describes most vertebrate systems, evolutionary transitions from GSD to TSD should be evolutionarily simple when appropriate selection occurs and incubation temperature varies, regardless of YY/WW viability costs.

Perhaps more intriguingly, under the special case where YY/WW individuals have equal fitness to other genotypes, we demonstrated that selection for TSD can produce a novel, evolutionarily stable system of mixed

sex-determining mechanisms. In the mixed-mechanism system, reached from either XX/XY or ZZ/ZW as a starting point, heterogametic individuals (ZW or XY) are male or female depending on incubation temperature (genotype-specific TSD), whereas homogametes of both type are opposite sexes (GSD; XX or WW females and ZZ or YY males). Whether a system evolves to pure TSD or the mixed-mechanism system depends largely on starting values of the threshold. In general, a system that starts closer in mutational distance to the mixed-mechanism system will move into that equilibrium. We propose that the selective forces maintaining this system include (i) selection for TSD in heterozygotes and (ii) frequency-dependent selection on sex maintaining an equilibrium ratio of males (ZZ + ZW) and females (ZW + WW). However, the mixed-mechanism system seemed to be less evolutionary stable than the pure TSD system. The variance in fitness was much higher in mixed-mechanism populations than in pure TSD populations, which likely contributed to the fact that individuals from a pure TSD population were able to invade a mixed-mechanism population but not *vice versa*. Although the invasion of pure TSD individuals was only common when invading at reasonably high frequencies into a mixed-mechanism population, this type of scenario is likely if species exist in many small, connected populations or demes. When demes of mixed-mechanism and pure TSD strategies exchange individuals through dispersal, the pure TSD strategy would easily spread through the population through successive invasion and fixation across demes.

That such a different view of evolution can arise by considering the underlying mechanisms highlights the power of thinking across levels of analysis, from proximate to ultimate (Laland *et al.*, 2011). The evolutionary path, in this case between sex-determining systems, was redefined when incorporating a generalized mechanistic model. Thus, a major strength of the model is its ability to incorporate both levels of analysis equally, simultaneously illuminating evolutionary processes as well as underlying mechanisms (Uller & Helanterä, 2011).

Models of the evolution of TSD typically leave the mechanistic underpinnings of SDMs unspecified, in effect assuming that mechanisms impose no limitations on phenotypic evolution (e.g. Van Dooren & Leimar, 2003; Schwanz & Proulx, 2008). Some of the predictions from these optimality models seem to be consistent with predictions using the generalized mechanistic model. For example, a fluctuating environment still selects against TSD (Pen *et al.*, 2010). In addition, the mechanistic model allows addressing novel questions, such as whether changing climates induce neutral turnover among SDMs (Grossen *et al.*, 2011). A fruitful extension of this research would be to further examine how the specified mechanism alters the evolutionary dynamics of SDMs in varying environmental and life historical conditions. In particular, if sexually antagonistic selection leads to male-beneficial genes aggregating on the Y chromosome (or female-beneficial genes on W), sex-reversed individuals (e.g. XX males or WW females) may have reduced fitness compared with heterogametic competitors, which may dramatically alter the evolutionary path.

Although most of the evolutionary outcomes of our model are clearly TSD, genetic variation also plays an important role in the evolved systems in a fashion similar to empirical examples. In particular, some ZZ (and XX) individuals develop as female at any temperature owing to having a genetically determined threshold that is greater than the peak male signal. Thus, in the model, this common form of TSD (female-mixed-female pattern; Harlow, 2004) is produced by novel genetic variation in the threshold, but, importantly, not by the evolutionary maintenance of a small number of ZW individuals in the population. In fact, the rapid loss of the W allele (or chromosome) suggests that the maintenance of a minority of ZW females is not evolutionarily stable.

The rapid transition from GSD to TSD and the stability of the evolved mean threshold have implications for the debate as to whether SDMs represent a continuum or discrete set of options (Valenzuela *et al.*, 2003; Sarre *et al.*, 2004; Ospina-Alvarez & Piferrer, 2008). The mechanisms modelled here allow a continuum of the effects of genotype versus temperature on sex determination. In fact, Quinn *et al.* (2011) explore the possibility that any place along the sex-determining continuum can exist, depending on the value of the male-determining threshold. We have shown here that the sex-determining system moves very quickly from GSD through intermediate forms to TSD when faced with selection. Thus, the continuum view has validity in our model for conceptualizing the underlying molecular mechanisms, whereas the discrete view reflects evolutionarily stable states along that continuum.

The results suggest new avenues of inquiry into SDMs and their evolutionary transitions in nature. First, a lingering question is whether the observed mixed-mechanism system exists in nature. In our model, the mixed-mechanism system cannot evolve if YY/WW individuals have as small as 5% reduction in fitness. Some animals with GSD are recorded to have viable YY/WW individuals (Devlin & Nagahama, 2002); however, a 5% decrease in viability or fertility would be difficult to detect empirically. Given the limited biology for which our model predicts the evolution of the mixed-mechanism system, it is not surprising that natural examples do not abound. In fish, where YY and WW individuals are more often viable and fertile than in amniotes, a dizzying diversity of genetic sex-determining systems exists (Volff & Schartl, 2001; Devlin & Nagahama, 2002; Penman & Piferrer, 2008). Given the frequency with which species are also sensitive to

temperature-dependent sex reversal at extreme temperatures (Devlin & Nagahama, 2002), perhaps some wild species exist with a stable system that matches the system predicted.

Second, our model may begin to provide some insight into the recent observation by Ezaz *et al.* (2009) that, in lizards, TSD species predominantly occur in clades containing ZZ/ZW species and not in clades with only XX/XY species. Our model displayed a directional bias in evolution under selection for TSD (evolving upwards more easily than downwards), suggesting that TSD may be more likely to evolve from an ancestral ZZ/ZW system than an ancestral XX/XY system. This bias is likely observed for two reasons: (i) because there is a natural boundary imposed by a threshold value of zero, it is more difficult for the mean threshold to evolve downwards when mutants cannot have a negative value compared with evolving upwards when the threshold can exceed a value of 1 and (ii) across intermediate starting thresholds, mutants with higher values of the threshold see gradual increases in the likelihood of sex reversal, whereas mutants with lower values do not see any increases initially until the very sudden and dramatic increase when crossing the lower genotypic curve (Fig. 3). These features must reflect any natural system with bell-shaped signal expression and incubation temperature distributions. However, their influence on the evolution of the threshold in real organisms likely depends on the relative heights of signal expression reached by the alternative genotypes and by the width of the reaction norms across temperatures relative to the variance in incubations temperatures. For example, if the variance in incubation temperature is low enough so that many values of τ lead to no sex reversal of either genotype in a GSD system, then biases to evolving upwards or downwards from these starting threshold levels will depend intrinsically on the mutational distance to where sex reversal of each genotype begins occurring. The potential for an evolutionary bias must be examined across a much wider range of model parameter values before concluding that it is important in explaining empirical patterns.

Third, the model makes predictions as to the patterns of extreme-temperature sex reversal in GSD species. We modelled a scenario where the most common incubation temperature coincided with peak expression of the male signal and extreme temperatures led to sex reversal of the genotype that is normally male (ZZ or XY). Sex reversal of the female-typical genotype (ZW or XX) at one extreme of temperature is possible in species where typical incubation temperatures are higher or lower compared with peak male signal expression (Quinn *et al.*, 2011); however, sex reversal of the female genotype at both high and low temperature extremes is not possible. We suggest that thresholds in natural systems will most often exist at levels where only the homogametic sex is sex-reversed due to selection against matings between heterogametic individuals. Evidence from several reptile and fish taxa supports these predictions (e.g. Sato *et al.*, 2005; Quinn *et al.*, 2007; Radder *et al.*, 2008). Greater inquiry across heterothermic taxa (e.g. lizards) would provide strong tests of the generalized model of SDMs as well as the pathway of evolutionary transitions predicted herein.

Acknowledgments

The authors would like to thank J. Graves, A. Quinn, S. Sarre and the Wildlife Genetics and Reptile Genomics Groups in the Institute for Applied Ecology, University of Canberra, for stimulating discussion regarding this research. The manuscript was improved by comments from the Kokkonut Theory Group, Jim Bull and an anonymous reviewer. L.E.S. was supported by a University of Canberra Postdoctoral Fellowship.

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Appendix 1
Examining alternative states

To explore the relative stability of the pure TSD and mixed-mechanism systems, we examined the invasion ability of TSD mutants (ZZ and $\tau = 0.9675$) into the observed mixed-mechanism populations, and the invasion ability of a sample of mixed-mechanism individuals (ZZ (12%); ZW (56%); WW (32%); $\tau = 0.5813$) into the observed TSD populations (changing the relative survival probability of WW to 1). Each invasion scenario was replicated 10 times.

To determine whether individual fitness varied between populations demonstrating the TSD and mixed-mechanism systems, we calculated the expected fitness of each of the 1000 genetic combinations (genotype at the major locus + τ value) of individuals at the end of the simulations. To calculate expected fitness, we imagined each genetic combination developing across the range of potential incubation temperatures. Thus, expected fitness of each genotypic combination (W_i) present at the 10 000th iteration was calculated in MATLAB as the sum of temperature-specific fitness values across a relevant range of incubation temperatures (T_{inc}) weighted by the probability of experiencing the specified incubation temperature:

$$W_i = \sum_{T_{\text{inc}}=-15}^{15} \Pr(T_{\text{inc}}) * W_{i,T_{\text{inc}}} \qquad (1)$$

Temperature-specific fitness values were the product of the probability of surviving a given T_{inc}, and the sum of reproductive gains through male and female function:

$$W_{i,T_{\text{inc}}} = \Pr(\text{Surv}|T_{\text{inc}}) * [m(T_{\text{inc}}) + f(T_{\text{inc}})] \qquad (2)$$

$\Pr(\text{Surv}|T_{\text{inc}})$ does not depend on genotype in this formulation because no genotypic-dependent variation

in survival was present at the end of the simulations (e.g. no WW present when viability was 0). In equation [2], $m(T_{inc})$, the reproduction gained through male function at a given temperature, is the product of the probability that the genetic combination will become a male at that temperature and the reproductive success gained by being a male at that temperature:

$$m(T_{inc}) = \Pr(\text{male}|\text{genos}, T_{inc}) * RS(\text{male}|T_{inc}) \quad (3a)$$

The reproduction gained through female function is a similar formulation:

$$f(T_{inc}) = (1 - \Pr(\text{male}|\text{genos}, T_{inc})) * RS(\text{female}|T_{inc}) \quad (3b)$$

Because reproductive success depends on the individual's RRS and on the RRS of same-sex reproductive competitors from other temperatures, we normalized RRS according to the mean RRS of same-sex individuals in the 10 000th iteration:

$$RS(male|T_{inc}) = RRS(\text{male}|T_{inc})/\overline{RRS}_m \quad (4a)$$

$$RS(\text{female}|T_{inc}) = RRS(\text{female}|T_{inc})/\overline{RRS}_f \quad (4b)$$

where $\overline{RRS}_f$ is the average RRS of real males in the 10 000th iteration and $\overline{RRS}_f$ is the average RRS of real females in the 10 000th iteration.

To visualize how the expected fitness above was driven by sex-specific effects, we additionally calculated expected fitness through male and female function separately as

$$W_{i,m} = \sum_{T_{inc}=-15}^{15} \Pr(T_{inc}) * \Pr(\text{Surv}|T_{inc}) * m(T_{inc}) \quad (5a)$$

for males, and

$$W_{i,f} = \sum_{T_{inc}=-15}^{15} \Pr(T_{inc}) * \Pr(\text{Surv}|T_{inc}) * f(T_{inc}) \quad (5b)$$

for females. Mean expected fitness for each sex was calculated as these summed values divided by the number of incubation temperatures that produced non-zero fitness sex-specific values. Hence, the mean expected fitness represents the fitness a genotypic combination could expect when developing as a male or female.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Evolutionary simulation code in the programming language R.
Data S2 Evolutionary simulation code in the programming language R.
Data S3 Evolutionary simulation code in the programming language R.
Data S4 Evolutionary simulation code in the programming language R.
Data S5 Evolutionary simulation code in the programming language R.
Data S6 Evolutionary simulation code in the programming language R.
Data S7 Evolutionary simulation code in the programming language R.
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Data S12 Evolutionary simulation code in the programming language R.

Received 12 February 2013; revised 23 August 2013; accepted 31 August 2013