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Climate-driven shifts in adult sex ratios via sex reversals: the type of sex determination matters

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Summary

Sex reversals whereby individuals of one genetic sex develop the phenotype of the opposite sex occur in ectothermic vertebrates with genetic sex-determination systems that are sensitive to extreme temperatures during sexual differentiation. Recent rises in global temperatures have led researchers to predict that sex reversals will become more common, resulting in the distortion of many populations' sex ratios. However, it is unclear whether susceptibility to climate-driven sex-ratio shifts depends on the type of sex determination that varies across species. First, we show here using individual-based theoretical models that XX/XY (male-heterogametic) and ZZ/ZW (female-heterogametic) sex-determination systems can respond differentially to temperature-induced sex reversals. Interestingly, the impacts of climate warming on adult sex ratio (ASR) depend on the effects of both genotypic and phenotypic sex on survival and reproduction. Second, we analyse the temporal changes of ASR in natural amphibian populations using data from the literature, and find that ASR shifted towards males in ZZ/ZW species over the past 60 years, but did not change significantly in XX/XY species. Our results highlight the fact that we need a better understanding of the interactions between genetic and environmental sex-determining mechanisms to predict the responses of ectotherms to climate change and the associated extinction risks.

Introduction

Global temperatures have been rising in recent decades, resulting in manifold effects on the Earth's ecosystems from shifts in phenology to altered distribution ranges to disease dynamics [1,2]. One particular biological process that is influenced by temperature is the development of the sexual phenotype in many ectothermic vertebrates. Temperature-dependent sex determination (TSD), whereby offspring sex is determined by post-fertilization environmental temperatures during a susceptible period of development, has been demonstrated in various species of fish and reptiles [3–5]. The concern that climate change may distort the sex ratio of TSD species has been raised repeatedly [5–7], as mounting evidence shows that unusually warm years yield hatchling sex ratios that are skewed towards the sex produced near the upper limit of tolerated incubation temperatures, which can result in biased sex ratio of the adult population [8]. It has even been proposed that past climate-change effects on sex ratios might have played a role in dinosaur extinctions [9].

Climate change, however, may also influence the sex ratios of populations with genetic sex-determination systems (GSD). In these species, sex is determined at fertilization by the sex chromosomes, such that heterogametic individuals (i.e. those with two different sex chromosomes, XY or ZW) develop into males in XX/XY systems and into females in ZZ/ZW systems [3]. Both systems are widespread in vertebrates, and in contrast to mammals (all male-heterogametic) and birds (all female-heterogametic), in ectotherms the type of sex determination is evolutionarily labile, differing even among closely related species of amphibians, reptiles and fish [3,4]. In these ectotherms, GSD can be overridden by unusually high or low temperatures during the sensitive period of ontogeny, resulting in sex-reversed individuals whose phenotypic sex differs from their genetic sex [10–14]. Theoretical models show that such sex reversals can lead to biased sex ratios [15–18], with far-reaching consequences for population viability [16,17,19] and the evolution of sex-determining mechanisms [14,20–23]. For example, sex reversals can either extirpate the population or boost its size [16,17], and they can propel evolutionary transitions from GSD to TSD [14,21,22] and between XX/XY and ZZ/ZW systems [23,24].

Intuitively, the impact of temperature-induced sex reversals on the population sex ratio may differ between the two major types of GSD: for example, if high temperatures masculinize genetic females, which is characteristic of amphibians and fish [5,10,11], in species with XX/XY systems the resulting XX males will mate with XX females, producing 100% XX offspring which may counter the sex-ratio distorting effect of masculinization. In contrast, in species with ZZ/ZW systems, masculinized ZW individuals will mate with ZW females, producing 25% male offspring (or 33% males if the WW genotype is not viable); thus this system may have lower capacity to compensate for masculinization by female-biased offspring sex ratios. Although a few previous theoretical models indicated that the two types of GSD might differ in their susceptibility to the effects of sex reversals on population demography [16,19], no study has yet formally compared the performance of the XX/XY and ZZ/ZW systems to test whether, and under what circumstances, they are differently affected by temperature-induced sex reversals.

In this study we employ a two-pronged approach to investigate climate-driven sex-ratio shifts in ectotherms with GSD. First, we develop an individual-based theoretical framework to investigate the effects of temperature-induced sex reversals on population sex ratios under various conditions and contrast these effects between the two types of GSD. Second, we compile empirical data from the literature to analyse the temporal changes of sex ratios in natural populations, and contrast these findings with the predictions of our model. We focus on amphibians, although our model is applicable to any taxon with GSD and naturally occurring masculinization. Out of the amphibian species studied so far, all have GSD [4,25] (but see [26]), and almost all can be experimentally masculinized with high temperatures (ca. above 30°C) during larval life, probably due to the temperature-dependence of aromatase (or its inhibitors) which converts androgens into estrogens [10,11]. It has long been suspected that reproductively functional sex-reversed individuals occur in natural amphibian populations [12,27]. A recent study found that 9% of genetically female adults were phenotypic males in a population of common frogs (*Rana temporaria*) and 17% of the genotyped clutches were all-female, likely resulting from the matings between two XX genotypes, i.e. a normal female and a masculinized (genotypically female but phenotypically male) individual [18]. While sex reversals are difficult to study in the field due to a general lack of genetic sexing methods in amphibians [18,28], phenotypic sex ratios of adults have been studied extensively in some species over the past decades. Therefore, we tested whether adult sex ratios (ASR) changed over the last 60 years in parallel with climate warming [29], whether this change differed between species with different GSD systems, and whether the empirical results can be explained by increasing masculinization rates according to our model.

Methods

Theoretical modelling

We modelled the effects of increasing masculinization rate using an individual-based model in a diploid population with overlapping generations and either XX/XY or ZZ/ZW sex determination. Our specific parameter values are explained in table S1 (electronic supplementary material 1). Each year, a maximum number of N_{max} offspring were allowed to complete metamorphosis; thereby we assumed that survival from eggs to metamorphosis is density-dependent due to limited carrying capacity of the environment. The actual number of newly metamorphosed offspring in each year was calculated as $N = \min(N_{max}; fert \times N_{female})$ where N_{female} is the number of adult females and *fert* is the average number of offspring each female can recruit in the absence of density-dependence (i.e. when larval density is low due to scarcity of breeding females). Metamorphs' survival during the first winter was independent of phenotypic sex, but we allowed for an effect of genotype according to the "unguarded sex chromosome hypothesis" such that heterogametic individuals (XY or ZW) could have reduced survival, due to rare mutations on the X or Z chromosome that we assumed to exert deleterious effects primarily during early ontogeny (table S1).

After surviving the first winter, the individuals turned into juveniles with an annual survival rate independent of both genotypic and phenotypic sex (table S1). When reaching the age of maturity, which was allowed to differ between phenotypic males and females, juveniles turned into adults with an annual survival rate independent of age and genotype but dependent on phenotypic sex. For simplicity, we assumed a fixed life span; all adults died upon reaching this age (table S1). Each year, adults participated in a single breeding event, during which the mother of each of the N offspring was chosen randomly out of all phenotypically female adults, while the fathers were chosen from among the phenotypically male adults according to their mating success (α) which was allowed to vary with genotype (table S1).

Each model was run with these baseline settings and without masculinization for 50 years to allow the population structure (age and sex ratios) to stabilize. Then, each model was run for a further 350 years, during which the probability of masculinization increased linearly with some stochasticity as $p_{masc} = b_{masc} \times t \pm sd_{masc}$ where b_{masc} is the year-to-year increase in the average probability of masculinization while sd_{masc} is its standard deviation. Each year, the sex of newly produced, genotypically female (XX, ZW and WW) offspring was reverted to the male phenotype by the probability p_{masc}. Thus, we assumed that with rising average temperatures, the chances of stochastic variation resulting in temperatures high enough to trigger sex reversal will increase as well. We assumed no individual variability in the likelihood of masculinization and we did not allow resistance to sex reversal to evolve because we were primarily interested in the effects over an evolutionarily short time span to facilitate comparison with available empirical data (see below). However, in the ZZ/ZW system, neofemales with the WW genotype (resulting from the matings between normal ZW females and sex-reversed ZW males) were either allowed to get masculinized with the same probability as ZW females, or not at all (table S1). Masculinized individuals were then allowed to reproduce according to their phenotype and produce gametes according to their genotype, with no epigenetic inheritance of sex reversal [14,30].

We ran the model with 32 different combinations of parameter settings (hereafter scenarios, see below), each repeated in 100 runs. We examined two alternative sex-specific life histories: one characterizing urodelans (i.e., the age of first reproduction and adult survival being similar in both sexes), whereas the other corresponding to anurans (i.e., males maturing earlier but experiencing higher mortality than females; see table S1). With each of these two life histories, the models were run with 16 scenarios (table S1), representing 4 different effects of genotypic sex (see panel rows in figure 1) combined with 4 different effects of phenotypic sex (see panel columns in figure 1). Specifically, the 4 different scenarios of genotypic sex effects assumed, respectively, that 1) genotypic sex has no effect on survival or masculinization, 2) the heterogametic sex suffers extra mortality early in life due to the "unguarded sex chromosome" effect, 3) the WW genotype is lethal, and 4) WW females cannot be masculinized. Each of these 4 scenarios was run with 4 different phenotypic sex effects, assuming that the mating success of masculinized individuals (i.e. phenotypic males with the XX, ZW or WW genotype) is 100%, 75%, or 1% compared to normal males, or linked to the Y or Z chromosomes (i.e. 50% in ZW males and 1% in WW and XX males).

For each scenario, we report the results by plotting the qualitative effects of increasing masculinization rate on the phenotypic ASR and the relative frequency of each genotype until p_{masc} reaches 1. From the same models, we statistically analysed the phenotypic ASR over the first 60 years for comparison with the empirical data (see below). For each scenario we extracted the regression slope of ASR change over 60 years from each run and calculated its 95% confidence interval for the two GSD systems. We compared the slope of ASR change between the XX/XY and ZZ/ZW system within each scenario by Welch-tests and corrected the p-values for false recovery rate. All models were run in R 3.1.0 [31]; the R code of our model is available in electronic supplementary material 2.

Empirical study

We compiled data on the ASR (proportion of males in the adult population) from the literature as described earlier [32]. We searched in Google Scholar for the terms "species name" and "sex ratio" for all amphibian species for which either male or female heterogamety has been demonstrated according to the Tree of Sex database [25]. We excluded studies where the authors stated or speculated that their data may not represent the population sex ratio, or when the methods were not described in enough detail to assess the adequacy of the study (for more details on collecting, filtering and validating the data, see [32]). We found data on natural populations for 39 species; the repeatability of ASR among populations within species was 0.56 [32]. However, long-term data were not available; for the vast majority of the studied populations, ASR has been reported for 1 or 2 years (maximum: 6 years). From each study we extracted ASR records for each year per population, keeping only the records with $N > 20$ individuals. We restricted our analyses to species for which there were at least 10 records and at least 10 years between the earliest and latest study year. If a study did not report annual ASR but provided average ASR over several (2–4) years, we assigned the midpoint of the study period as study year of that record.

The data that fit these criteria totalled 125 records of 6 species (2 anurans and 4 urodelans) from 51 studies conducted between 1953 and 2011. Since the ASR data for each species came from several populations, we collected data for 3 potentially confounding variables that may influence

ASR: latitude, altitude, and morph. As amphibian demography and life history can vary along geographical gradients [33], we extracted the latitude and altitude of the study location for each ASR record. As urodelans can be either metamorphic or paedomorphic, and the two morphs can differ in sex ratio [34], we categorized morph for each record as the presence or absence of paedomorphic adults (i.e. sexually mature individuals that retain larval somatic traits) in the population. Our dataset is available in electronic supplementary material 3.

To analyse the relationship between ASR and time expressed as the number of years since 1950, we used a mixed-effects modelling framework. By estimating random intercepts and random slopes, we allowed the 6 species to differ in average ASR and in the slope of the change of ASR with time, respectively. As fixed effects we included time, GSD, and the time × GSD interaction to test whether species with XX/XY and ZZ/ZW systems differed in the slope of ASR change over the years. We also included the potentially confounding effects of latitude and altitude as covariates, and morph as a fixed factor. This approach assumes that each ASR record is a random sample taken from the pool of each species' all populations at various points in time and space, testing whether time (a proxy for climate warming) or geographical gradients have a systematic effect on amphibian sex ratios (see [35] for a similar approach applied to turtle sex ratios).

We implemented the mixed-effects model by Bayesian approach, using the R package 'MCMCglmm' [36]. We ran 4 MCMC chains with inverse Wishart priors ($V = 1$, $nu = 0.002$; [36]), each with 7,000,000 iterations, a thinning interval of 500 and a burn-in of 2,000,000 iterations, yielding 10,000 samples per chain, from which we calculated the posterior distribution of all parameter estimates (fixed and random effects). We tested the convergence of model parameters among the chains using the Gelman-Rubin statistic; the potential scale reduction factor was 1 in all cases, showing that model convergence was appropriate. We report the parameter estimates (posterior means) and corresponding 95% credibility intervals (CI) from the first chain. Autocorrelation was < 0.02 for all estimated parameters, and there was no heteroscedasticity by sample size (figure S1 in electronic supplementary material 1).

Results

Theoretical modelling

As the rate of masculinization increased over several hundred years, the model predicted increasingly male-biased ASR in most scenarios (figure 1). ASR started to shift later if the sex-reversed individuals had relatively high mating success (figure 1). When the masculinized individuals reproduced just as well as normal males, the normal male genotype went extinct in most scenarios by the time masculinization rate reached 100%, and the initial GSD transitioned into a TSD system in which all individuals have female genotypes (XX, or WW and ZW) and phenotypic males are produced solely by temperature-induced masculinization (figure 1*a*,*e*,*i*,*m*). WW lethality sped up the ASR shift while slowing down the change of genotype frequencies (figure 1*i*,*j*,*k*,*l*). When WW females could not be masculinized, ZW females and ZZ males were replaced by WW females and ZW males, respectively, thus leading to a switch from ZZ/ZW to XX/XY system (figure 1*m*,*n*,*p*). Similar changes occurred, albeit more slowly, when the mating success of masculinized individuals was 75% compared to normal males (figure 1*b*,*f*,*j*,*n*). In contrast, when their mating success was close to zero, both systems shifted rapidly to male-biased ASR with little change in the genotype frequencies (figure 1*c*,*g*,*k*,*o*). For the XX/XY system, this latter scenario is equivalent to the cases when male mating success is Y-linked, whereas for the ZZ/ZW system, Z-linked mating success predicted the same, albeit slower, trends as did the scenarios with 75% mating success of masculinized individuals (figure 1*d*,*h*,*l*,*p*). The corresponding "urodelan" and "anuran" scenarios yielded similar results (figure S2 in electronic supplementary material 1).

Over the first 60 years after masculinization rate had started to increase, both GSD systems shifted toward significantly more male-biased ASR in most scenarios (figure 2; table S2 and figure S3 in electronic supplementary material 1). This shift was significantly stronger in the XX/XY than in the ZZ/ZW system in 7 of the 8 scenarios when male mating success was Y/Z linked (table S2, figure 2). In contrast, the shift was significantly stronger in the ZZ/ZW than in the XX/XY system in 3 scenarios (table S2): when masculinized individuals had high mating success (100% or 75% of normal males) and the WW genotype was lethal (figure 2*i*,*j*), or when masculinized individuals had 100% mating success and heterogametic offspring suffered extra mortality early in life (figure 2*e*). The slope of ASR change never differed between the two GSD systems when the masculinized individuals had very low mating success (table S2, figure 2).

Empirical study

The change of ASR over the years differed significantly between species with different GSD (table 1). The two species with female heterogamety (ZZ/ZW) shifted significantly towards more male-biased ASR over time (table 2, figure 3), with an average increase of 0.4 % per year (CI: 0.03, 0.77). In contrast, the 4 species with male heterogamety (XX/XY) showed no significant change in ASR over the years (table 2, figure 3), with an average decrease of 0.17 % per year (CI: -0.43, 0.08). This observed pattern, i.e. increasing male bias in ZZ/ZW and no change in XX/XY, is qualitatively consistent with the theoretical scenarios where $\alpha = 1$ and the WW genotype is lethal or the “unguarded” heterogametic offspring suffer extra mortality (table S2, figure 2*e*,*i*, figure S3*e*,*i*).

ASR did not vary systematically with latitude and altitude, while it was significantly lower in populations containing paedomorphic adults (table 1). The difference among species or between the two GSD systems was not attributable to spatial differences in the rate of climate warming (figure S4 in electronic supplementary material 1).

Discussion

Our most important novel finding, as demonstrated by the theoretical models and indirectly supported by the empirical results, is that the XX/XY and ZZ/ZW systems can differ remarkably in their susceptibility to climate change. Our model showed that the direction and extent of this difference varies with the mating success of sex-reversed individuals, the nature of genotype-dependent differences in offspring viability, and the ability of WW individuals to sex-reverse. The empirical data showed that the ASR shifted towards males in ZZ/ZW but not in XX/XY species of temperate-zone amphibians over the past decades while climate has been warming in their habitats. Comparing these changes of ASR (figure 3) to the patterns predicted by our model (figure 2), the theoretical scenario that qualitatively best matches the empirical data is the case where masculinized individuals can reproduce as well as normal males but offspring survival is genotype-dependent, due to either WW lethality or extra mortality of the “unguarded” heterogametic offspring. In both of these scenarios, the potential to dampen the effects of increasing masculinization rate by the production of female-biased offspring is constrained in the ZZ/ZW system compared to the XX/XY system, because early-life mortality of the genotypes is female-biased in the former but not in the latter. To explore whether these conditions are met in natural populations, and to be able to predict the impacts of climate change, detailed knowledge has to be accumulated on each species regarding the type of sex-determination system, the reproductive success of sex-reversed individuals, and the effects of genotypic sex on offspring mortality. As most of this information is lacking for the majority of amphibian species and ectotherms in general, this challenging task will hinge on the development of genetic sexing methods [28].

Our model corroborates several findings from earlier theoretical studies which showed that sex ratios can vary with climate warming in species with GSD via temperature-induced sex reversals. Firstly, if masculinized individuals are not handicapped in reproduction, higher than 50% masculinization rates lead to the extinction of the male genotype, and phenotypic males are produced solely by temperature-induced masculinization [15]. Thus, the system switches from GSD to TSD even in the absence of selection for TSD, i.e. without any sex-specific fitness benefit favouring sex-specific developmental temperatures [23]. Secondly, if sex-reversed individuals have reduced reproductive success, masculinization shifts ASR towards males more rapidly, while genotypic sex ratios change more slowly [17]. Thirdly, WW lethality can affect the outcome [22] by saving the Z chromosome from extinction but rapidly shifting the ASR towards males [15]. Finally, if WW females are resistant to masculinization, for example due to accumulation of male-antagonistic alleles on the W chromosome [22], increasing masculinization rates trigger a switch between GSD systems from ZZ/ZW to XX/XY [20].

Notably, these changes occurred in our models over several hundreds of years; and similarly, theoretical studies that allowed the sex-determination system to evolve via mutations (which was not permitted in our models) found such switches after hundreds or thousands of years [21–23]. Given the rapidity of contemporary environmental change [29], the question remains whether species can adapt fast enough [7,14]. The latest evolutionary transition between XX/XY and ZZ/ZW systems in amphibians is dated ca. 1 MYA [37], and the youngest neo-sex chromosome, presumably representing a switch from a mixed GSD-TSD system to a pure XX/XY system, has been documented from a lineage that diverged ca. 10 KYA [26]. Nevertheless, several empirical findings suggest ample potential for adaptation. Even individuals with the same sex chromosomes under identical environmental conditions can vary in their propensity to develop into male or female, as pointed out by a recent review [38] and by studies reporting that extreme temperatures do not always induce sex reversal in all individuals [11]. It is not yet known whether the individual variation in sex-reversal propensity is heritable, although TSD species exhibit considerable heritability in the sex-determination threshold [39], and in a GSD lizard the offspring of sex-reversed (ZZ) mothers were found to have greater temperature sensitivity, suggesting heritable variation in susceptibility to feminization [14]. Heritable variation in sex-reversal propensity would allow sex-determination thresholds to evolve, and this may fundamentally change the effects of climate change as well [21,22,24]. Thus, future models should allow for adaptation when comparing the responses of XX/XY and ZZ/ZW systems.

A further limitation of our theoretical study is that it did not allow for phenotypically plastic adjustments of parental or offspring behaviour in choice of thermal environment. For example, many species have been responding to climate change by breeding earlier or changing nest depth [6]; it is unclear whether or not such behavioural adjustments can counter-balance the effects of climate change on sex ratios [6–8]. Because the thermal environment has critical effects on growth and developmental rates as well as on the exposure to pathogens, predators and competitors, the consequences of changing the time or place of offspring development are non-trivial and can be counter-intuitive. For example, a shift of spawning to earlier dates in a fish species has led to increasingly colder environment for the offspring at the time of sex determination, because water temperatures rose more slowly in early spring than later in the season [40]. Future theoretical studies of sex reversals should account for such effects of phenotypic plasticity.

Over 60 years of climate warming, our model predicted small, although ecologically still relevant increases in ASR, averaging an additional 0.08% of males per year (table S2 in electronic supplementary material 1), which adds up to an extra ca. 5% of males in total over six decades. The data observed in nature showed relatively rapid changes in ASR, adding up to an extra 21-27% of males in ZZ/ZW species over 60 years (i.e. the slope values in Table 2 multiplied by 60), although these estimates inevitably had relatively high uncertainty. Changes of such magnitude are significant from evolutionary and conservationist viewpoints alike, as sex ratios have far-reaching consequences for sexual and parental behaviours, demography, and population viability [41–44]. For example, male-biased ASR may reduce effective population size and the population's intrinsic rate of increase [7,8], intensify sexual competition including sexual coercion and male-male contest that can be harmful or even fatal to females [45–47], or induce homosexual behaviours [47].

It is unlikely that the difference we found between the two GSD systems was a by-product of spatially heterogeneous climate change, because the increase of maximum temperatures at the study locations was similar in XX/XY and ZZ/ZW species. Also, since we controlled for geographical variability and morph differences in our analyses, the contrasting temporal trends we found are unlikely to be artefacts of different populations being studied in different years, although among-population heterogeneity is a likely cause for the high uncertainty of our slope estimates. Due to the lack of long-term datasets, the number of species in our analysis is very small especially for the ZZ/ZW species, so further evidence will be needed to corroborate the pattern we found. Nevertheless, the two ZZ/ZW species have little in common in terms of life history or phylogeny that would make them stand apart from the four XX/XY species, suggesting that the difference we found is related to the sex-determination system. It is also notable that in two additional ZZ/ZW species for which we found data with at least 10 years between the earliest and latest study year (2 records for each species), ASR increased from 41% in 1958-1959 to 64% in 2002-2003 in *Duttaphrynus melanostictus* and from 34% in 1981-1984 to 42% in 1997-2002 in *Crinia signifera* [48–51].

We caution that the observed changes of ASR do not necessarily result from global warming; direct evidence for the role of climate change and sex reversals in these empirical trends should come from long-term studies of sex ratios in various age groups within natural populations coupled with extensive genetic analyses. Until such data become available, it has to be born in mind that the temporal changes of ASR may be influenced by factors unrelated to temperature. For example, pesticides widely used in agricultural practice [52,53] and pharmaceuticals and other xenoestrogens associated with urbanization [54,55] have the potential to interfere with sexual development and induce sex reversal, and species can differ substantially in their susceptibility to sex reversal by the same chemicals [28]. Furthermore, because both temperature and chemicals can disrupt the same endocrine pathway that is responsible for sexual differentiation [11,52], and high temperatures can exacerbate pesticide toxicity [52,53], chemical pollution and climate change may interact to affect sex ratios in complex ways. Such synergistic effects may be difficult to predict unless both chemical and temperature effects are considered simultaneously in theoretical and empirical studies.

Sex-determining mechanisms exhibit astonishing diversity among taxa [3], and evidence is accumulating that this diversity contributes profoundly to variation in demographic traits such as ASR [32] and evolutionary processes such as adaptive radiation [56] and genome evolution [57]. Our results highlight the fact that the type of GSD can also influence the species' susceptibility to sex-ratio shifts driven by environmentally induced sex reversals. Thus, understanding the interactions between genetic and environmental effects on sex determination will be essential for predicting the among-species variation in vulnerability and adaptability to environmental changes such as climate warming. Well-informed predictions about these issues, in turn, may prove crucial in the future for the conservation of ectothermic species.

Data Accessibility. The datasets supporting this article have been uploaded as part of the Supplementary Material.

Authors' Contributions. VB conceived of the study, collected and analysed the data, and wrote the manuscript. VB, SK and EN designed the modelling study; SK and EN programmed the models. SK, EN, AL and TS critically reviewed the manuscript. All authors gave final approval for publication.

Competing Interests. We have no competing interests.

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Table 1. Linear mixed-effects model for empirical ASR in relation to time (years since 1950) and GSD (genetic sex-determination system) in 6 amphibian species. ASR is expressed as percentage of males in the adult population to avoid very small model parameter values.

Model parameters	Posterior mean	95% CI Lower bound	95% CI Upper bound	*p*
Intercept (ZZ/ZW, metamorphic)	55.140	39.030	70.760	0.001
Latitude	-0.205	-0.621	0.240	0.348
Altitude	-0.003	-0.007	0.001	0.123
Morph (difference of paedomorphic)	-17.860	-26.310	-9.185	<0.001
GSD (difference of XX/XY from ZZ/ZW)	-0.140	-19.310	21.210	0.964
Time (slope in ZZ/ZW species)	0.403	0.018	0.766	0.036
Time × GSD (difference of slope)	-0.581	-1.010	-0.103	0.016

Table 2. Annual change of empirical ASR in 6 amphibian species with different GSD (genetic sex-determination system), estimated from the model in table 1. ASR is expressed as percentage of males in the adult population to avoid very small model parameter values.

			95% CI	
Species	GSD	Slope	Lower bound	Upper bound
Ambystoma tigrinum	ZZ/ZW	0.350	0.030	0.671
Bufo bufo	ZZ/ZW	0.455	0.134	0.777
Ichthyosaura alpestris	XX/XY	-0.085	-0.356	0.186
Lissotriton vulgaris	XX/XY	-0.168	-0.376	0.041
Triturus cristatus	XX/XY	-0.212	-0.498	0.074
Rana temporaria	XX/XY	-0.234	-0.499	0.031

Figure 1. Model-predicted changes over 350 years in ASR (proportion of phenotypic males) and relative frequencies of genotypes, in scenarios with no sex difference in maturation age and adult survival. The width of each curve shows the 95% confidence band from 100 runs. The average rate of masculinization increases from zero by 0.003 each year; α denotes the mating success of masculinized individuals (i.e. phenotypic males with the XX, ZW or WW genotype) relative to normal males. Note that the parameter settings for the XX/XY system are the same in the scenarios "α = 0.01" and "α Y-linked" (see table S1).

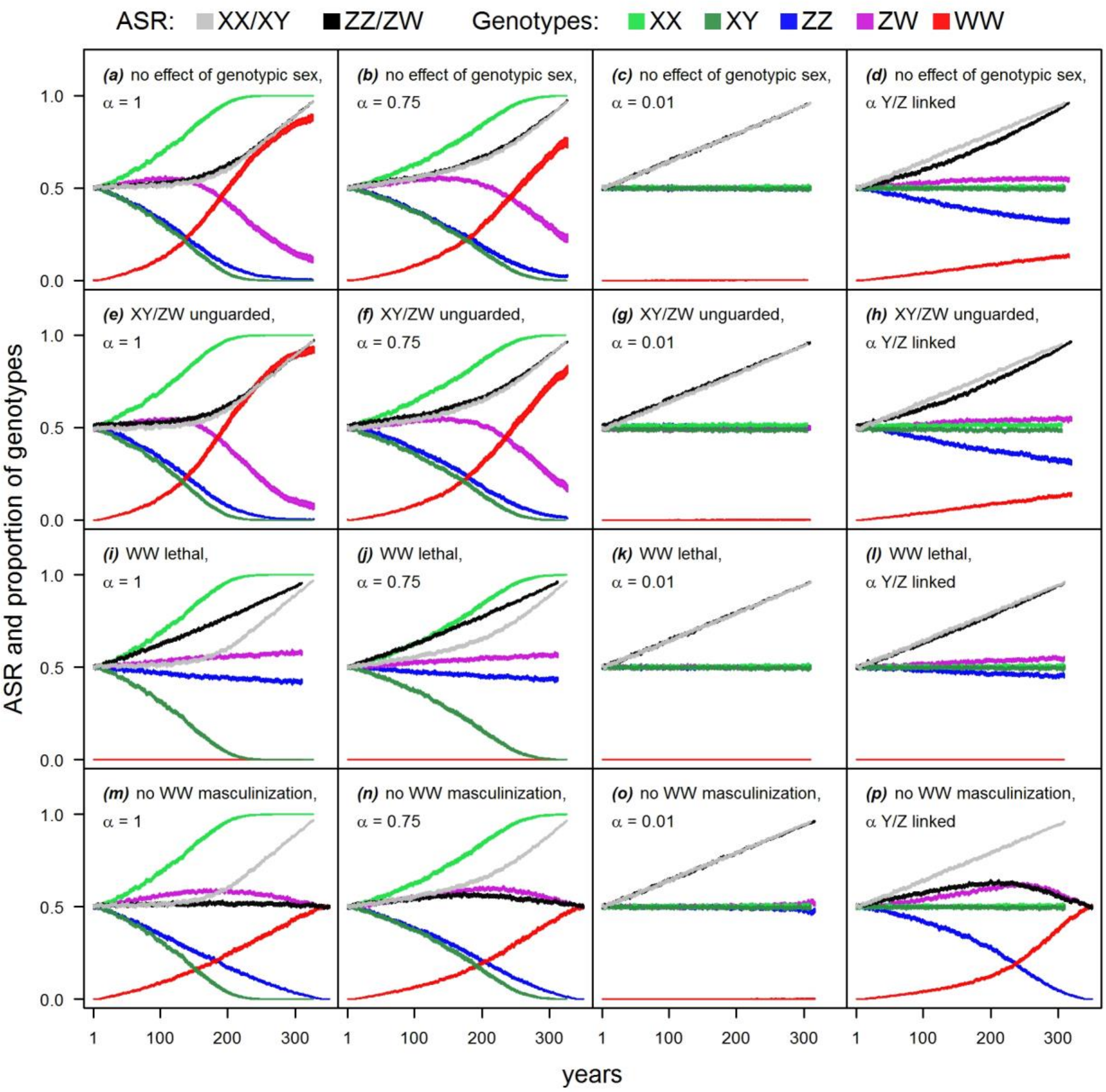


Figure 2. Model-predicted changes of adult sex ratio over the first 60 years in the scenarios with no sex difference in maturation age and adult survival. Black and grey polygons show the 95% confidence bands of the slopes in ZZ/ZW and XX/XY systems, respectively. Asterisks mark the scenarios in which the slopes differ significantly between the two systems ($p < 0.05$ after correction for false discovery rate). The average rate of masculinization increases from zero by 0.003 each year; α denotes the mating success of masculinized individuals relative to normal males. Note that the parameter settings for the XX/XY system are the same in the scenarios "α = 0.01" and "α Y-linked" (see table S1).

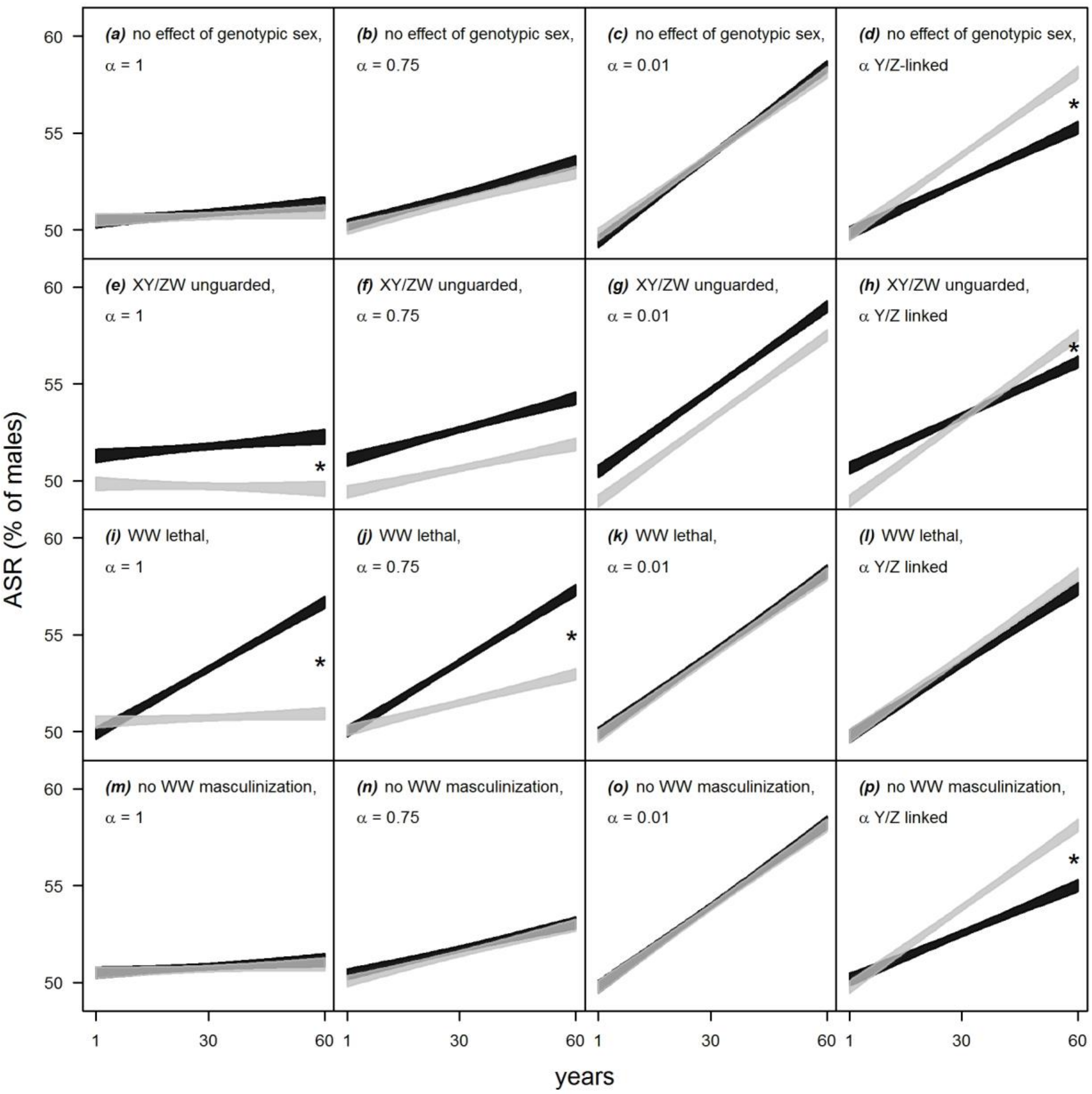
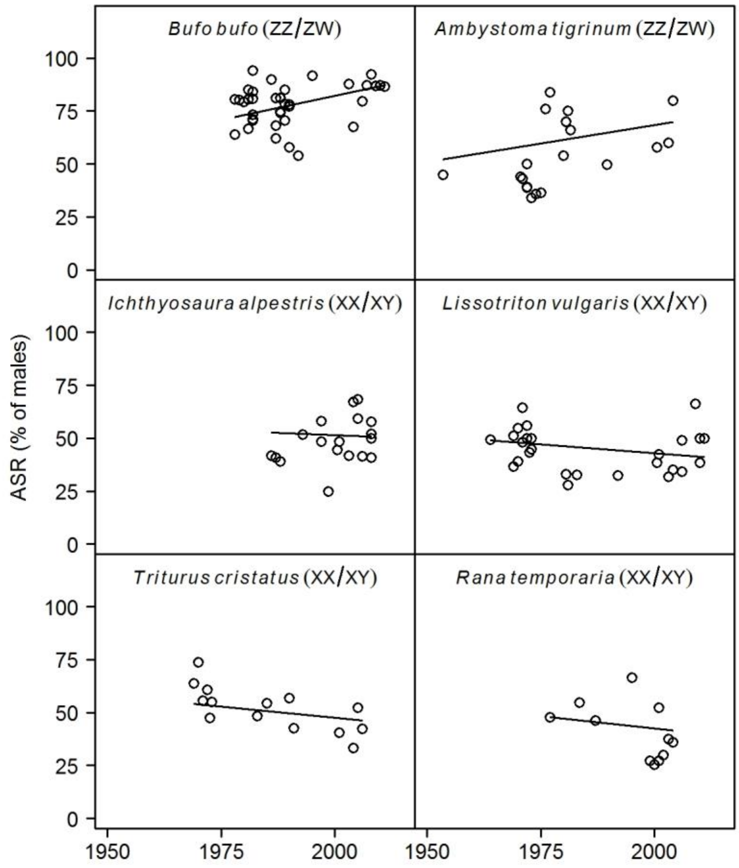


Figure 3. Empirical adult sex ratios over time in amphibians with either XX/XY or ZZ/ZW sex-determination systems. Slopes are fitted from the model in table 1; species are ordered by slope from most positive to most negative.



Supplementary material for
Veronika Bókony, Szilvia Kövér, Edina Nemesházi, András Liker, Tamás Székely (2017): "Climate-driven shifts in adult sex ratios via sex reversals: the type of sex determination matters", Phil. Trans. R. Soc. B.
doi: 10.1098/rstb.2016.0325

This document (**Supplement 1**: Additional tables and figures) contains the following items:

Table S1: Definition, notation, and value of each parameter used in our models, with justification and references

Table S2: Model predictions for each scenario: the slope of change in ASR in XX/XY and ZZ/ZW systems, and the slope's difference between the two systems with FDR-corrected p-values

Figure S1: Empirical adult sex ratios in amphibian populations, in relation to sample size

Figure S2: The "anuran" scenarios (with sex-dependent maturation age and adult survival) corresponding to the "urodelan" scenarios in figure 1

Figure S3: The "anuran" scenarios (with sex-dependent maturation age and adult survival) corresponding to the "urodelan" scenarios in figure 2

Figure S4: The rate of climate warming observed at the study locations of the 6 species

Further supplementary material available as separate files:

Supplement 2: R codes of the sex-reversal models (ESM2.R)

Supplement 3: Empirical data of adult sex ratios in amphibian populations (ESM3.xls)

Table S1. Definition, notation, and value of each parameter used in our models, with justification and references. Parameter values highlighted in bold were fixed in all runs.

Parameter	Notation	Value	Justification
Number of adult females in the initial population (equals the number of adult males)	*NF0*	**100**	Because population size is often small in amphibians, and small populations are likely of highest concern to conservation, we start with a population of 200 adults. The initial number of offspring and juveniles is min(*N_max, NF0×fert*), and sex ratio is set to 1:1 in all age classes. The equilibrium proportions of sexes and age classes will stabilize during the burn-in period.
Initial sex ratio of offspring and juveniles (proportion of the heterogametic sex)	*XY0; WZ0*	**0.5**	
Maximum annual number of metamorphosed offspring	*N_max*	**2000**	Larval survival is density-dependent [48].
Average annual number of metamorphosed offspring per female	*fert*	**200**	Median clutch size: 400 [49]; density-independent survival from egg to metamorphosis: 0.5 [48].
Annual survival rate of juveniles	*phi_juv*	**0.4**	Mean of published values [48,50–54].
Maximum life span	*lifespan*	**12**	Median longevity of amphibians in the AnAge database [55].
Initial probability of masculinization	*m_masc0*	**0**	Assuming no masculinization before 1970 and an increase to 9% masculinization by 2000 [16] gives *b_masc*=0.003; *sd_masc* reflects the SD of temperature anomalies in the Northern Hemisphere between 1970 and 2000 [56].
Yearly increase of masculinization probability	*b_masc*	**0.003**	
SD of masculinization probability	*sd_masc*	**0.01**	
Number of years without masculinization	*t_burn_in*	**50**	The burn-in period allows the population structure to stabilize.
Number of years with increasing masculinization	*t_Max*	**350**	Following the population until masculinization rate reaches 100%.

Number of model runs per parameter setting	*n_runs*	**100**	Each scenario takes several hours to run.
Probability of masculinization in WW individuals relative to WZ individuals	*p_rel_WW_masc*	1	WW females can be masculinized [22,57].
		0	WW females cannot be masculinized if Z-linked genes are needed for male development and/or the W chromosome has accumulated male-antagonistic alleles [20,58].
Survival from metamorphosis to first spring	*phi_XX*, *phi_XY;* *phi_ZZ*, *phi_WZ*, *phi_WW*	all = 0.3	Mean of published values [48,59]. No difference in survival between sex-reversed and normal individuals [22,60,61].
		phi_WZ = 0.29, *phi_XY* = 0.29	Extra mortality due to the “unguarded sex chromosome” [62]; based on the frequency of human X-linked recessive disorders [63].
		phi_WW = 0	WW individuals are not viable in some species [22,64].
Age of first reproduction in males and females	*mat_m*, *mat_f*	both = 2	Males and females mature at the same age on average [65,66].
		mat_m = 2, mat_f = 3	Males mature earlier than females [67–69].
Annual adult survival rate in males and females	*phi_m*, *phi_f*	both = 0.5	Mean of published values (e.g. [50,70–72]). No difference in survival between males and females [70,71,73] or between sex-reversed and normal individuals [60,61].
		phi_m = 0.4, *phi_f* = 0.6	Adult survival is lower in phenotypic males than in females [72–74].

Mating success of masculinized individuals relative to normal males	*alpha_XX; alpha_WZ, alpha_WW*	1	Masculinized individuals reproduce as successfully as normal males [22,75].
		0.75	Reproductive success of sex-reversed individuals reduced by 25% compared to normal males [61].
		0.01	Sex-reversed individuals are sterile [22].
		alpha_XX=0.01; *alpha_WZ*=0.5, *alpha_WW*=0.01	Male reproductive success is linked to fertility genes on the Y or Z chromosome [58,76,77].

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Table S2. Model predictions for each scenario: the slope of change in ASR over 60 years and its 95% confidence interval in XX/XY and ZZ/ZW systems, and the slope's difference between the two systems with FDR-corrected *p*-values. Slopes and differences that differ significantly from zero are highlighted in bold. ASR is expressed as percentage of males in the adult population to avoid very small model parameter values.

Model scenarios			ASR slope (95% CI)		GSD difference		
Life history	Genotype effects	*α*	XX/XY	ZZ/ZW	t	*p*	Graph
"urodelan"	none	1	0.007 (-0.002, 0.016)	**0.016 (0.006, 0.026)**	-1.23	0.352	Fig.2a
		0.75	**0.049 (0.041, 0.057)**	**0.055 (0.046, 0.064)**	-1.00	0.483	Fig.2b
		0.01	**0.142 (0.131, 0.152)**	**0.153 (0.145, 0.161)**	-1.79	0.147	Fig.2c
		Z/Y-linked	**0.142 (0.131, 0.152)**	**0.092 (0.083, 0.101)**	7.15	**<0.001**	Fig.2d
	XY/ZW unguarded	1	-0.004 (-0.014, 0.006)	**0.017 (0.005, 0.028)**	-2.74	**0.019**	Fig.2e
		0.75	**0.042 (0.033, 0.05)**	**0.054 (0.043, 0.064)**	-1.77	0.147	Fig.2f
		0.01	**0.145 (0.137, 0.153)**	**0.144 (0.135, 0.154)**	0.14	0.930	Fig.2g
		Z/Y-linked	**0.145 (0.137, 0.153)**	**0.093 (0.084, 0.102)**	8.39	**<0.001**	Fig.2h
	WW lethal	1	0.007 (-0.002, 0.016)	**0.115 (0.106, 0.123)**	-16.90	**<0.001**	Fig.2i
		0.75	**0.049 (0.041, 0.057)**	**0.124 (0.116, 0.132)**	-12.97	**<0.001**	Fig.2j
		0.01	**0.142 (0.131, 0.152)**	**0.142 (0.133, 0.15)**	-0.04	0.967	Fig.2k
		Z/Y-linked	**0.142 (0.131, 0.152)**	**0.129 (0.119, 0.139)**	1.69	0.160	Fig.2l
	no WW masculinization	1	0.007 (-0.002, 0.016)	**0.011 (0.002, 0.02)**	-0.56	0.709	Fig.2m
		0.75	**0.049 (0.041, 0.057)**	**0.045 (0.037, 0.054)**	0.65	0.659	Fig.2n
		0.01	**0.142 (0.131, 0.152)**	**0.144 (0.134, 0.153)**	-0.29	0.881	Fig.2o
		Z/Y-linked	**0.142 (0.131, 0.152)**	**0.082 (0.074, 0.091)**	8.84	**<0.001**	Fig.2p
"anuran"	none	1	**0.017 (0.005, 0.028)**	**0.031 (0.019, 0.042)**	-1.68	0.160	Fig.S3a
		0.75	**0.037 (0.027, 0.047)**	**0.053 (0.041, 0.065)**	-1.99	0.102	Fig.S3b
		0.01	**0.131 (0.121, 0.141)**	**0.128 (0.117, 0.139)**	0.35	0.862	Fig.S3c
		Z/Y-linked	**0.131 (0.121, 0.141)**	**0.081 (0.069, 0.093)**	6.36	**<0.001**	Fig.S3d
	XY/ZW unguarded	1	0.005 (-0.006, 0.015)	**0.024 (0.014, 0.034)**	-2.63	**0.022**	Fig.S3e
		0.75	**0.046 (0.035, 0.057)**	**0.04 (0.029, 0.051)**	0.76	0.597	Fig.S3f
		0.01	**0.132 (0.122, 0.142)**	**0.126 (0.115, 0.137)**	0.80	0.590	Fig.S3g
		Z/Y-linked	**0.132 (0.122, 0.142)**	**0.074 (0.063, 0.086)**	7.44	**<0.001**	Fig.S3h
	WW lethal	1	**0.017 (0.005, 0.028)**	**0.097 (0.086, 0.108)**	-10.05	**<0.001**	Fig.S3i
		0.75	**0.037 (0.027, 0.047)**	**0.124 (0.114, 0.134)**	-11.96	**<0.001**	Fig.S3j
		0.01	**0.131 (0.121, 0.141)**	**0.129 (0.118, 0.14)**	0.25	0.883	Fig.S3k
		Z/Y-linked	**0.131 (0.121, 0.141)**	**0.111 (0.1, 0.121)**	2.73	**0.019**	Fig.S3l
	no WW masculinization	1	**0.017 (0.005, 0.028)**	**0.016 (0.002, 0.029)**	0.12	0.930	Fig.S3m
		0.75	**0.037 (0.027, 0.047)**	**0.055 (0.043, 0.066)**	-2.32	0.050	Fig.S3n
		0.01	**0.131 (0.121, 0.141)**	**0.138 (0.127, 0.149)**	-0.95	0.499	Fig.S3o
		Z/Y-linked	**0.131 (0.121, 0.141)**	**0.071 (0.06, 0.081)**	8.09	**<0.001**	Fig.S3p

Figure S1. Empirical adult sex ratios in amphibian populations with XX/XY (empty symbols) and ZZ/ZW (filled symbols) sex-determination systems, in relation to sample size (note the logarithmic scale on the X axis). A mixed model assuming that variance decreases with increasing sample size did not fit the data better than the model in table 1 (difference in the deviance information criterion: 0.006); the two models yielded qualitatively identical results.

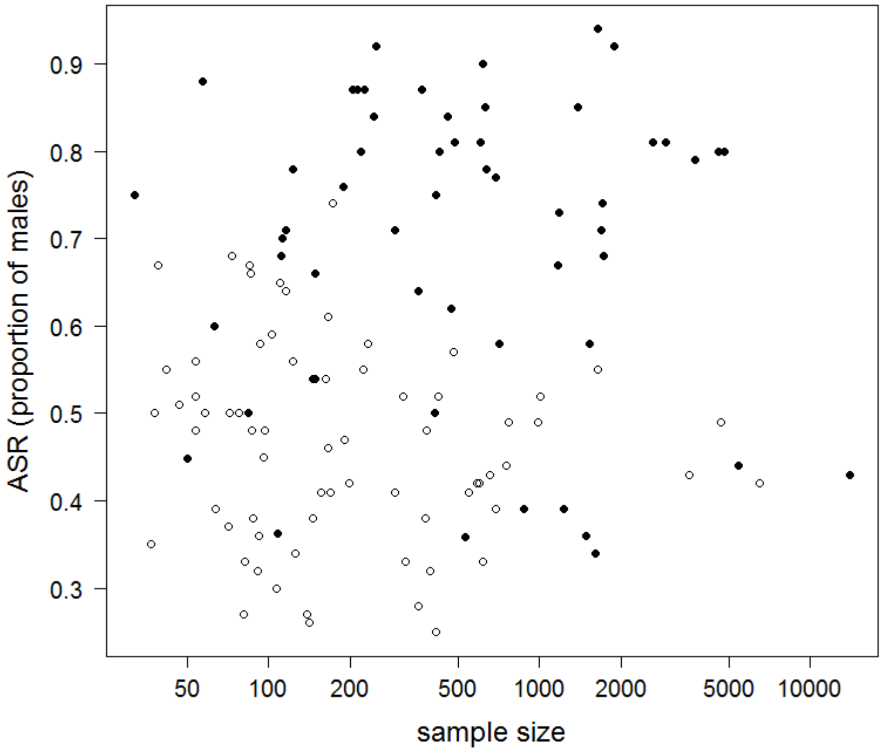


Figure S2. The "anuran" scenarios (with sex-dependent maturation age and adult survival) corresponding to the "urodelan" scenarios in figure 1: model-predicted changes over 350 years in ASR (proportion of phenotypic males) and relative frequencies of genotypes. The width of each curve shows the 95% confidence band from 100 runs. The average rate of masculinization increases from zero by 0.003 each year; α denotes the mating success of masculinized individuals (i.e. phenotypic males with the XX, ZW or WW genotype) relative to normal males. Note that the parameter settings for the XX/XY system are the same in the scenarios "α = 0.01" and "α Y-linked" (see table S1).

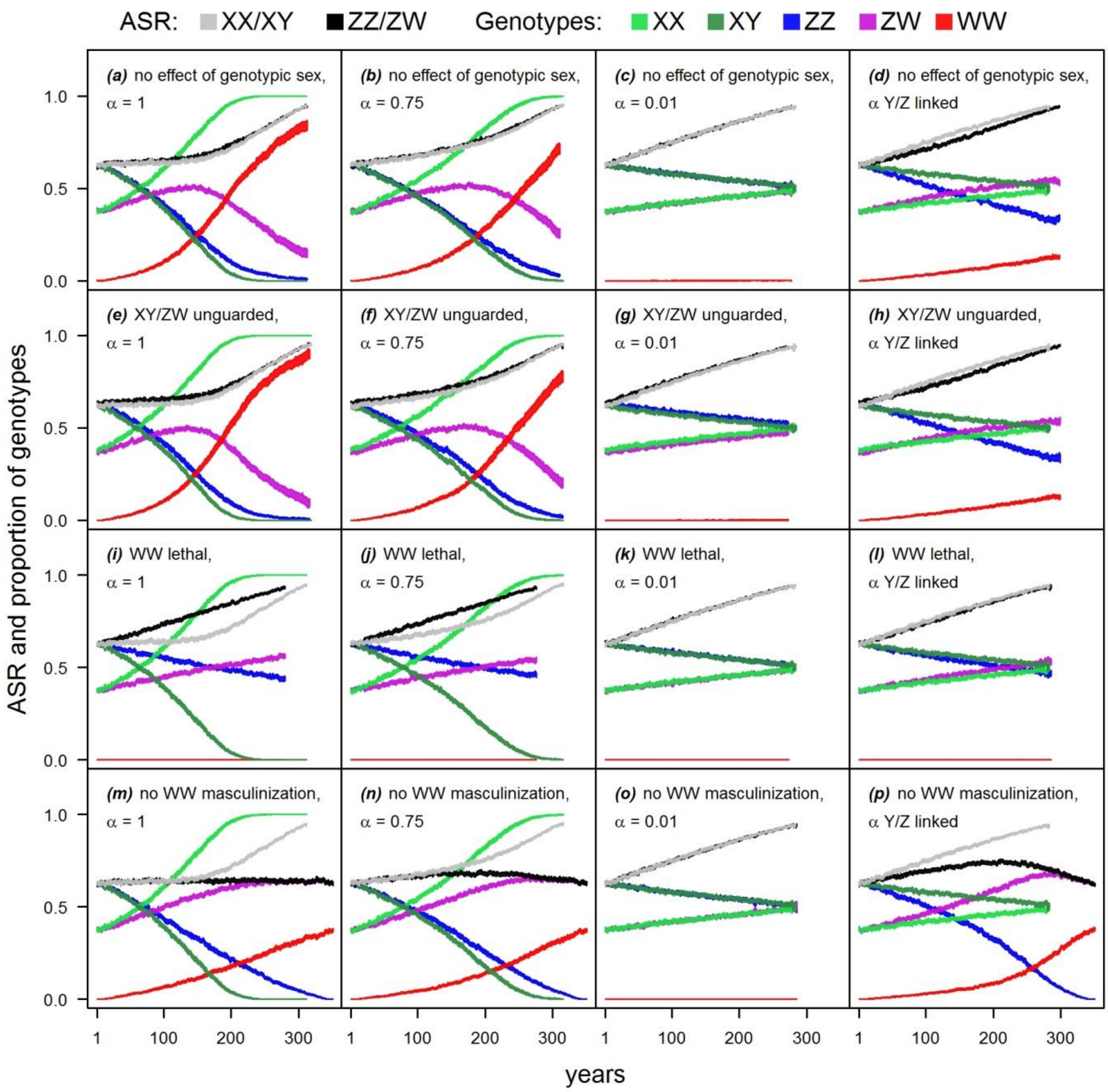


Figure S3. The “anuran” scenarios (with sex-dependent maturation age and adult survival) corresponding to the “urodelan” scenarios in figure 2: model-predicted changes of adult sex ratio over the first 60 years . Black and grey polygons show the 95% confidence bands of the slopes in ZZ/ZW and XX/XY systems, respectively. Asterisks mark the scenarios in which the slopes differ significantly between the two systems ($p < 0.05$ after correction for false discovery rate). The average rate of masculinization increases from zero by 0.003 each year; α denotes the mating success of masculinized individuals relative to normal males. Note that the parameter settings for the XX/XY system are the same in the scenarios “α = 0.01” and “α Y-linked” (see table S1).

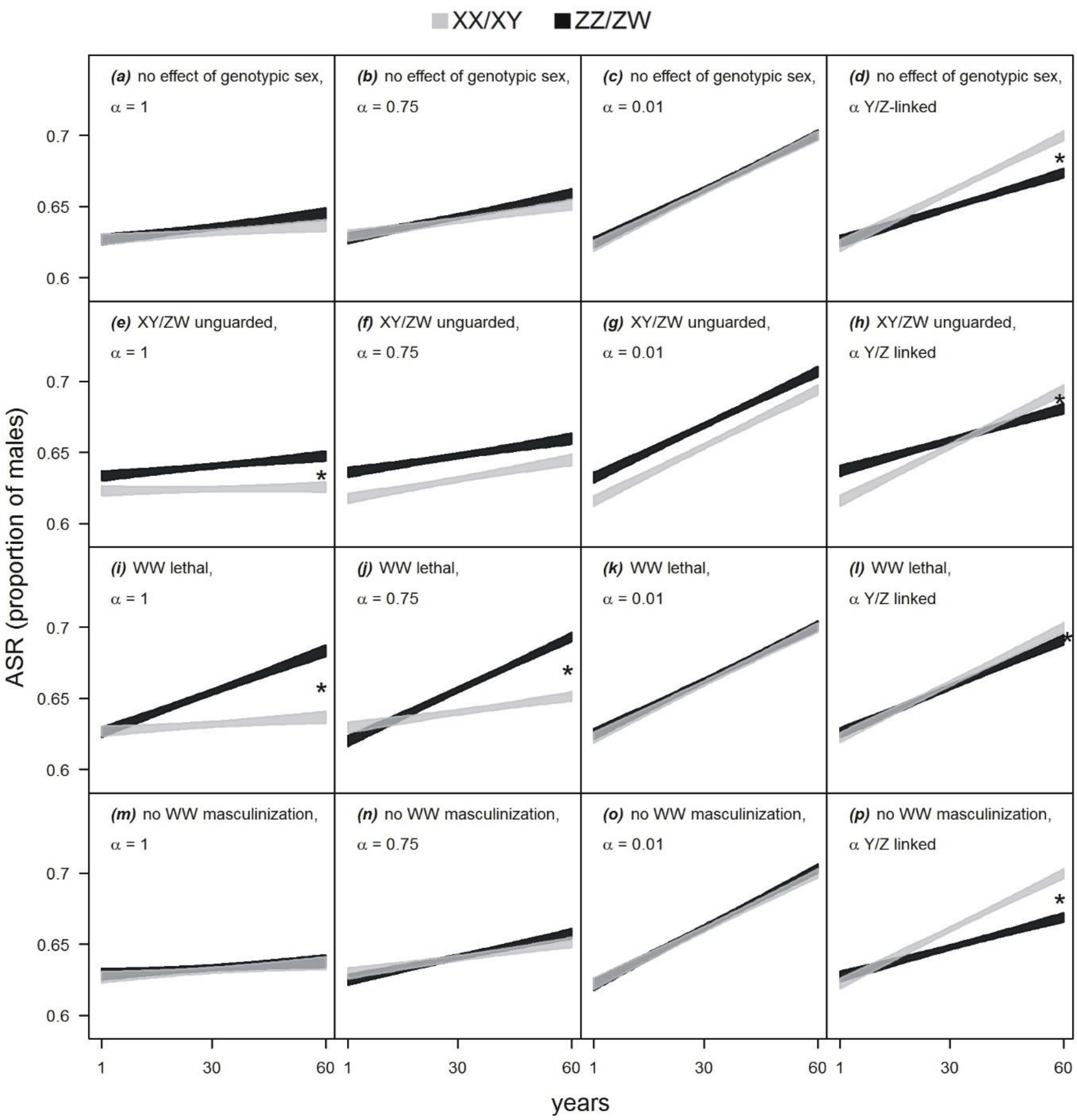
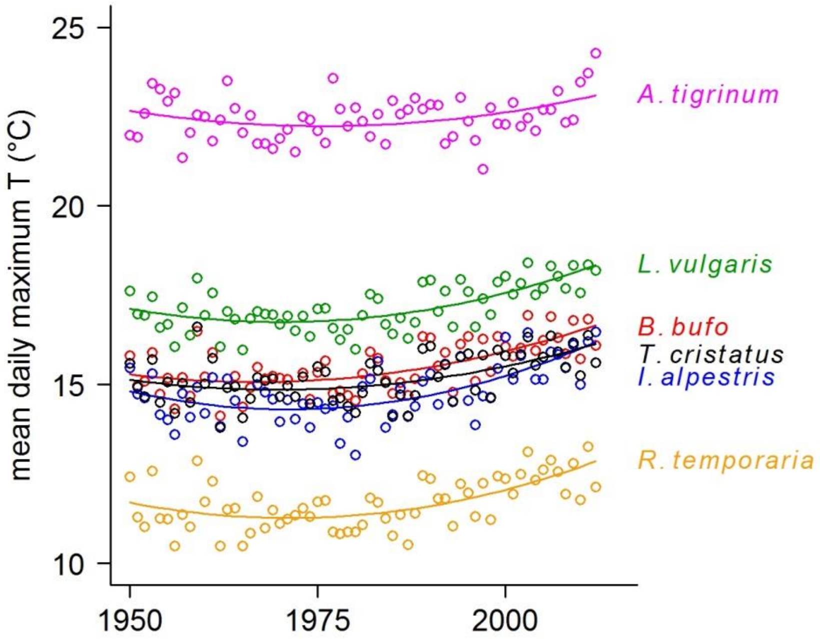


Figure S4. The rate of climate warming observed at the study locations of the 6 species.



To check whether the empirical differences we found in ASR change between GSD systems was attributable to spatial heterogeneity in the rate of climate change, we tested whether extremely high temperatures became more abundant over the past 60 years at the study locations and whether this warming was similar across species. To this end, we collected data on the monthly averages of daily maximum temperatures for the geographical coordinates of each ASR study between 1950 and 2012 from the CRU database [78], and for each species and each year we calculated the mean of monthly values excluding the winter months, as the developmental period of the study species occurs between spring and autumn. Because global climate has been warming more rapidly in the more recent decades [33,78], we fitted second-order polynomial curves to the temperature data while allowing different slopes per GSD (fixed factor) and species (random factor). This model showed that the interaction between time and GSD was not significant ($F_{2,368} = 1.01$, $p = 0.366$), meaning that the rate of warming at the locations of the studied populations did not differ significantly between XX/XY and ZZ/ZW systems. Then we fitted a similar model by removing GSD and using species identity as a fixed factor; this model also showed that the interaction between time and species identity was not significant ($F_{10,360} = 1.43$, $p = 0.166$), meaning that the 6 species were unlikely to experience different rates of climate warming at the study sites during the study period. Therefore, our finding that ASR changed differently over the years in XX/XY and ZZ/ZW species cannot be explained by geographically heterogeneous rates of climate change.

Reference:

78. Harris, I., Jones, P. D., Osborn, T. J. & Lister, D. H. 2014 Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. Int. J. Climatol. 34, 623–642. (doi:10.1002/joc.3711)