

**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.**

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.csv)

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author and source are credited.*

**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)	<i>NF0</i>	<b>100</b>	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 $\min(N\_max, NF0 \times fert)$ , 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)	<i>XY0; WZ0</i>	<b>0.5</b>	
Maximum annual number of metamorphosed offspring	<i>N_max</i>	<b>2000</b>	Larval survival is density-dependent [48].
Average annual number of metamorphosed offspring per female	<i>fert</i>	<b>200</b>	Median clutch size: 400 [49]; density-independent survival from egg to metamorphosis: 0.5 [48].
Annual survival rate of juveniles	<i>phi_juv</i>	<b>0.4</b>	Mean of published values [48,50–54].
Maximum life span	<i>lifespan</i>	<b>12</b>	Median longevity of amphibians in the AnAge database [55].
Initial probability of masculinization	<i>m_masc0</i>	<b>0</b>	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	<i>b_masc</i>	<b>0.003</b>	
SD of masculinization probability	<i>sd_masc</i>	<b>0.01</b>	
Number of years without masculinization	<i>t_burn_in</i>	<b>50</b>	The burn-in period allows the population structure to stabilize.
Number of years with increasing masculinization	<i>t_Max</i>	<b>350</b>	Following the population until masculinization rate reaches 100%.

Number of model runs per parameter setting	$n\_runs$	<b>100</b>	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	<i>alpha_XX</i> ; <i>alpha_WZ</i> , <i>alpha_WW</i>	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].
		<i>alpha_XX</i> =0.01; <i>alpha_WZ</i> =0.5, <i>alpha_WW</i> =0.01	Male reproductive success is linked to fertility genes on the Y or Z chromosome [58,76,77].

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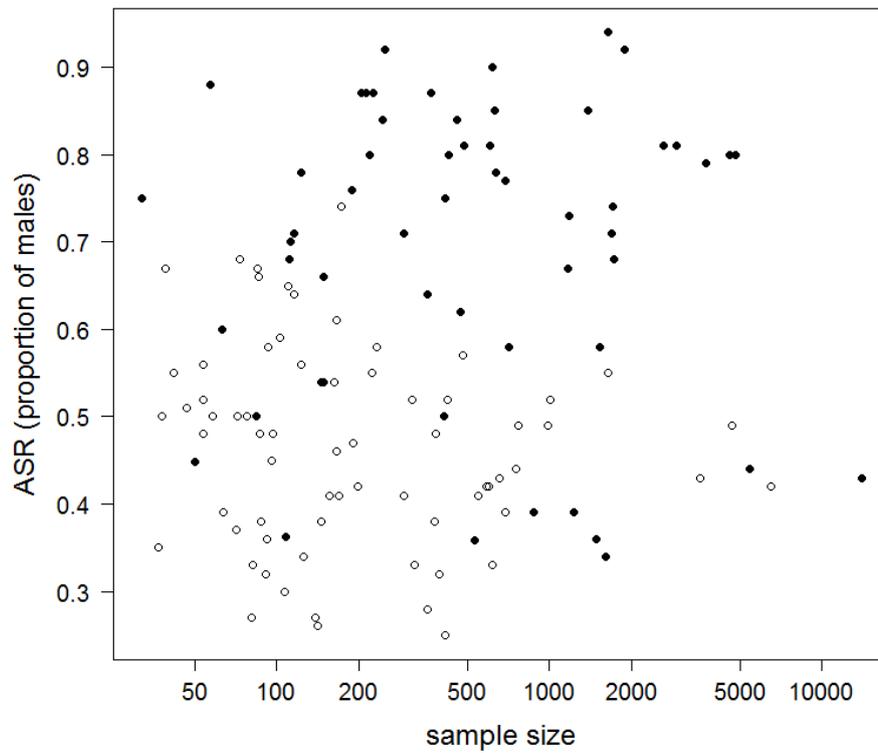
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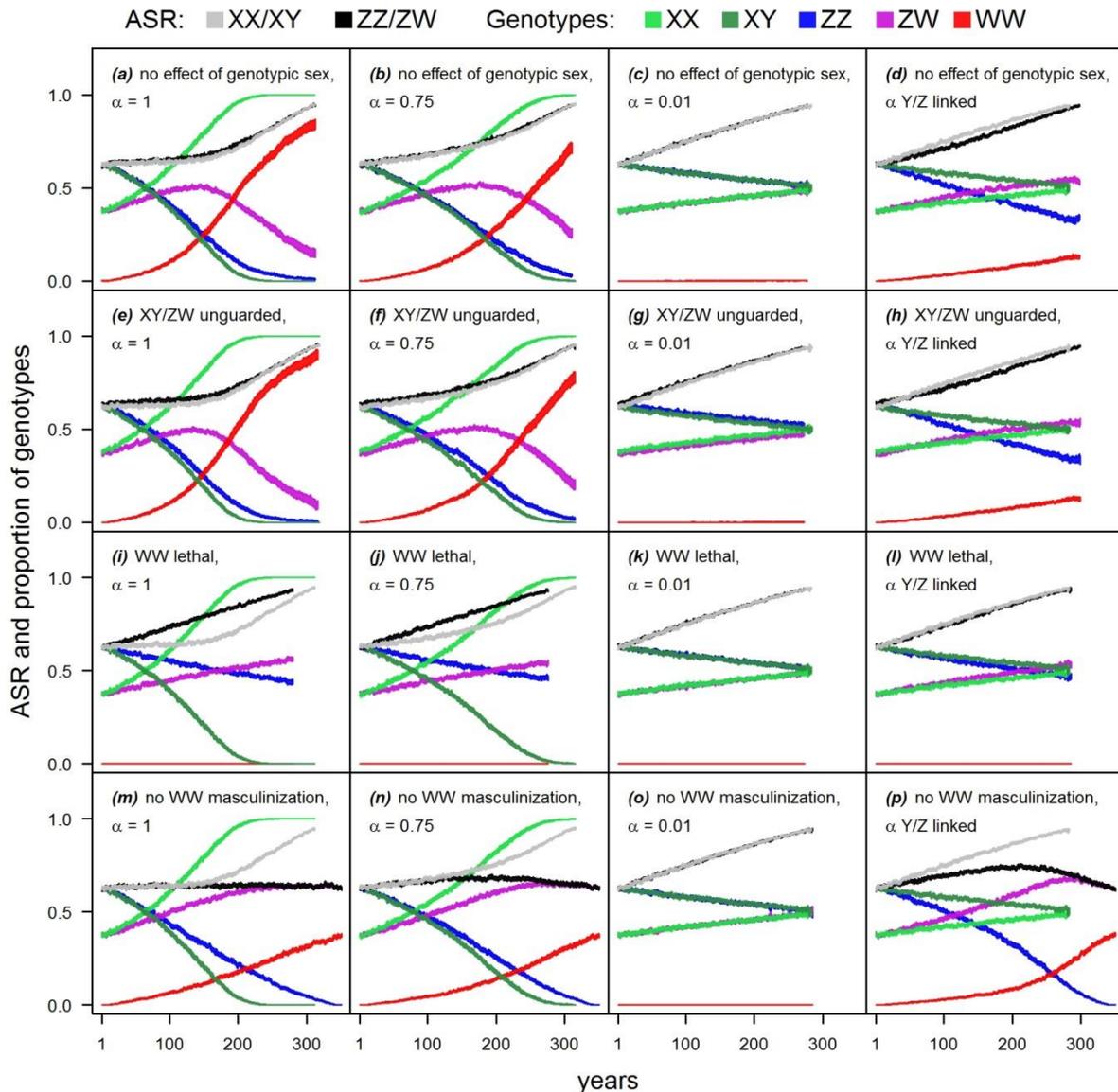
**Table S2.** Model predictions for each scenario: the slope of change in ASR over 60 years and its 95% confidence interval in XX/XY and ZZ/ZW systems, and the slope's difference between the two systems with FDR-corrected *p*-values. Slopes and differences that differ significantly from zero are highlighted 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		Graph
Life history	Genotype effects	<i>a</i>	XX/XY	ZZ/ZW	t	<i>p</i>	
"urodelan"	none	1	0.007 (-0.002, 0.016)	<b>0.016 (0.006, 0.026)</b>	-1.23	0.352	Fig.2a
		0.75	<b>0.049 (0.041, 0.057)</b>	<b>0.055 (0.046, 0.064)</b>	-1.00	0.483	Fig.2b
		0.01	<b>0.142 (0.131, 0.152)</b>	<b>0.153 (0.145, 0.161)</b>	-1.79	0.147	Fig.2c
		Z/Y-linked	<b>0.142 (0.131, 0.152)</b>	<b>0.092 (0.083, 0.101)</b>	7.15	< <b>0.001</b>	Fig.2d
	XY/ZW unguarded	1	-0.004 (-0.014, 0.006)	<b>0.017 (0.005, 0.028)</b>	-2.74	<b>0.019</b>	Fig.2e
		0.75	<b>0.042 (0.033, 0.05)</b>	<b>0.054 (0.043, 0.064)</b>	-1.77	0.147	Fig.2f
		0.01	<b>0.145 (0.137, 0.153)</b>	<b>0.144 (0.135, 0.154)</b>	0.14	0.930	Fig.2g
		Z/Y-linked	<b>0.145 (0.137, 0.153)</b>	<b>0.093 (0.084, 0.102)</b>	8.39	< <b>0.001</b>	Fig.2h
	WW lethal	1	0.007 (-0.002, 0.016)	<b>0.115 (0.106, 0.123)</b>	-16.90	< <b>0.001</b>	Fig.2i
		0.75	<b>0.049 (0.041, 0.057)</b>	<b>0.124 (0.116, 0.132)</b>	-12.97	< <b>0.001</b>	Fig.2j
		0.01	<b>0.142 (0.131, 0.152)</b>	<b>0.142 (0.133, 0.15)</b>	-0.04	0.967	Fig.2k
		Z/Y-linked	<b>0.142 (0.131, 0.152)</b>	<b>0.129 (0.119, 0.139)</b>	1.69	0.160	Fig.2l
	no WW masculinization	1	0.007 (-0.002, 0.016)	<b>0.011 (0.002, 0.02)</b>	-0.56	0.709	Fig.2m
		0.75	<b>0.049 (0.041, 0.057)</b>	<b>0.045 (0.037, 0.054)</b>	0.65	0.659	Fig.2n
		0.01	<b>0.142 (0.131, 0.152)</b>	<b>0.144 (0.134, 0.153)</b>	-0.29	0.881	Fig.2o
		Z/Y-linked	<b>0.142 (0.131, 0.152)</b>	<b>0.082 (0.074, 0.091)</b>	8.84	< <b>0.001</b>	Fig.2p
"anuran"	none	1	<b>0.017 (0.005, 0.028)</b>	<b>0.031 (0.019, 0.042)</b>	-1.68	0.160	Fig.S3a
		0.75	<b>0.037 (0.027, 0.047)</b>	<b>0.053 (0.041, 0.065)</b>	-1.99	0.102	Fig.S3b
		0.01	<b>0.131 (0.121, 0.141)</b>	<b>0.128 (0.117, 0.139)</b>	0.35	0.862	Fig.S3c
		Z/Y-linked	<b>0.131 (0.121, 0.141)</b>	<b>0.081 (0.069, 0.093)</b>	6.36	< <b>0.001</b>	Fig.S3d
	XY/ZW unguarded	1	0.005 (-0.006, 0.015)	<b>0.024 (0.014, 0.034)</b>	-2.63	<b>0.022</b>	Fig.S3e
		0.75	<b>0.046 (0.035, 0.057)</b>	<b>0.04 (0.029, 0.051)</b>	0.76	0.597	Fig.S3f
		0.01	<b>0.132 (0.122, 0.142)</b>	<b>0.126 (0.115, 0.137)</b>	0.80	0.590	Fig.S3g
		Z/Y-linked	<b>0.132 (0.122, 0.142)</b>	<b>0.074 (0.063, 0.086)</b>	7.44	< <b>0.001</b>	Fig.S3h
	WW lethal	1	<b>0.017 (0.005, 0.028)</b>	<b>0.097 (0.086, 0.108)</b>	-10.05	< <b>0.001</b>	Fig.S3i
		0.75	<b>0.037 (0.027, 0.047)</b>	<b>0.124 (0.114, 0.134)</b>	-11.96	< <b>0.001</b>	Fig.S3j
		0.01	<b>0.131 (0.121, 0.141)</b>	<b>0.129 (0.118, 0.14)</b>	0.25	0.883	Fig.S3k
		Z/Y-linked	<b>0.131 (0.121, 0.141)</b>	<b>0.111 (0.1, 0.121)</b>	2.73	<b>0.019</b>	Fig.S3l
	no WW masculinization	1	<b>0.017 (0.005, 0.028)</b>	<b>0.016 (0.002, 0.029)</b>	0.12	0.930	Fig.S3m
		0.75	<b>0.037 (0.027, 0.047)</b>	<b>0.055 (0.043, 0.066)</b>	-2.32	0.050	Fig.S3n
		0.01	<b>0.131 (0.121, 0.141)</b>	<b>0.138 (0.127, 0.149)</b>	-0.95	0.499	Fig.S3o
		Z/Y-linked	<b>0.131 (0.121, 0.141)</b>	<b>0.071 (0.06, 0.081)</b>	8.09	< <b>0.001</b>	Fig.S3p

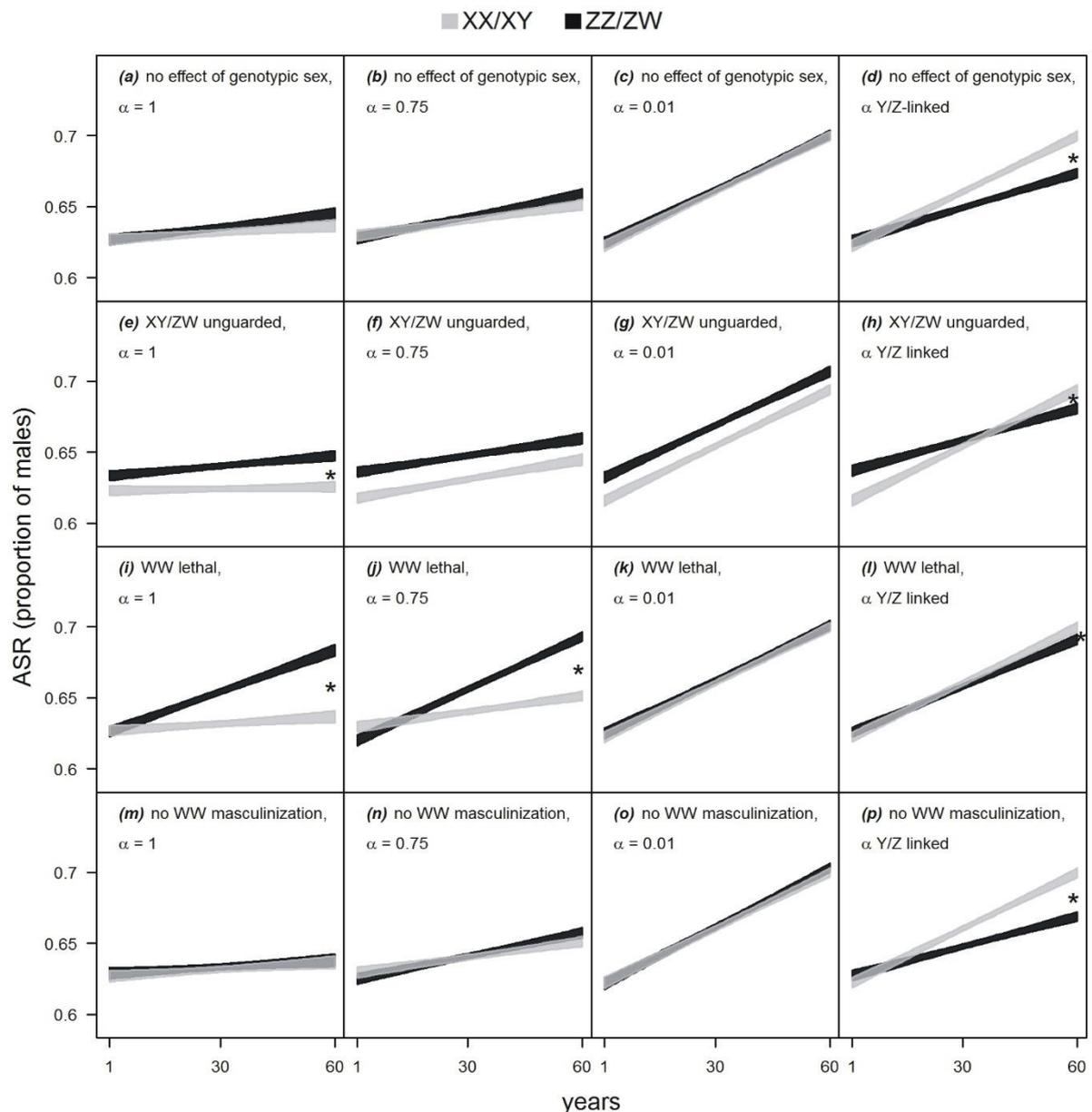
**Figure S1.** Empirical adult sex ratios in amphibian populations with XX/XY (empty symbols) and ZZ/ZW (filled symbols) sex-determination systems, in relation to sample size (note the logarithmic scale on the X axis). A mixed model assuming that variance decreases with increasing sample size did not 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.



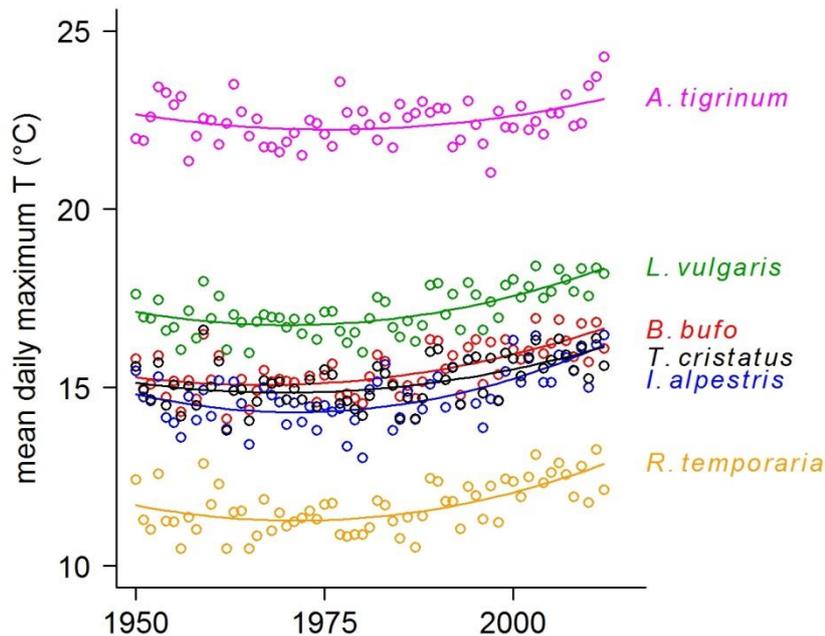
**Figure S2.** The “anuran” scenarios (with sex-dependent maturation age and adult survival) corresponding to the “urodelan” scenarios in figure 1: model-predicted changes over 350 years in ASR (proportion of phenotypic males) and relative frequencies of genotypes. The width of each curve shows the 95% confidence band from 100 runs. The average rate of masculinization increases from zero by 0.003 each year;  $\alpha$  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 “ $\alpha = 0.01$ ” and “ $\alpha$  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;  $\alpha$  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 “ $\alpha = 0.01$ ” and “ $\alpha$  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)