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Further Model Details

Life cycle

The life cycle of the model follow a simple structure. Figure S2 shows a life cycle graph[1] corresponding to the life history stages and the transitions between them for females in the lowland population. The disks denote the discrete life history stages: $i1...i5$ are the non-breeding females of ages 1 to 5 years old, where $i \in \{E,M,L\}$ indicates the birth date category. B_2 and $B_{>2}$ are the females in the breeding population, aged 2 or older, respectively. Thus, there are $3\times5+2=17$ different female life history stages. The life history of males is not shown, but follows a similar pattern except that there is no effect of birth date on their vital rates.

Non-breeding females of birth date category i and age j encounter one of three fates: they either enter the breeding population one year later with probability $(1-\mu)\alpha_{ij}$, where $(1-\mu)$ is the yearly survival probability and α_{ij} the probability of entering the breeding population conditional on survival; or, with probability $(1-\mu)(1-\alpha_{ij})$ they survive to age $j+1$ but do not enter the breeding population; or, with probability μ they die.

Females in the breeding population produce F_j ($j=2$ or >2) daughters per year, of which on average 1/3 enter birth date category i at (non-breeding) age 1. Each year, breeding females die with probability μ.

The life cycle of females from the higland population (not shown) is identical to that of lowland females, except for an additional stage of 3-year old breeding females.

Estimation of life history parameters

Clutch size and adult mortality rate

See table S2 for estimates of age-specific and population-specific frequency distributions of clutch sizes based on field studies of the two focal populations during 2000-2007.

Age at first breeding

Estimates of the conditional (on survival) probabilities α_{ij} of entering the breeding population at age j for females of birth date category i were inferred using an ML approach from the numbers of females of known year and timing of birth and age at first reproduction ($N = 73$ and $N = 131$, lowland and highland population, respectively; Table S2). Since no non-breeding females of 5 years or older were observed, we set $\alpha_{i5}=1$. Given unknown (α_{i2}, α_{i3}, α_{i4}) and known adult mortality rate μ, the expected frequencies u_{ij} of age-at-first breeding j or death for a given birth date category i equals:

$$\begin{aligned} u_{i2} &= \alpha_{i2} \\ u_{i3} &= (1-\mu)(1-\alpha_{i2})\alpha_{i3} \\ u_{i4} &= (1-\mu)^2(1-\alpha_{i2})(1-\alpha_{i3})\alpha_{i4} \\ u_{i5} &= (1-\mu)^3(1-\alpha_{i2})(1-\alpha_{i3})(1-\alpha_{i4}) \\ u_{i\dagger} &= 1-\sum_{j=2}^{j=5} u_{ij} \end{aligned} \qquad \text{(A1)}$$

The initial cohort size at age 2 is given by an unknown N_{i2}, and the observed counts of first breeders at age j are given by n_{ij} (Table S2). Then N_{i2} can be estimated from the counts and the estimated adult mortality rate $\hat{\mu}$ as

$$\hat{N}_{i2} = \sum_{j=2}^{5} n_{ij} / (1-\hat{\mu})^{j-2}. \qquad \text{(A2)}$$

Since the probabilities u_{ij} determine a multinomial distribution for a fixed i, the α_{ij} can be estimated for each i by maximizing with respect to (α_{i2}, α_{i3}, α_{i4}) the multinomial log-likelihood function

$$L(\alpha_{i2},\alpha_{i3},\alpha_{i4}) = \sum_{j=2}^{5} n_{ij}\log u_{ij} + (\hat{N}_{i2} - \sum_{j=2}^{5} n_{ij}\log u_{ij})\log(1-\sum_{j=2}^{5} u_{ij}). \quad \text{(A3)}$$

Numerical maximization was carried out with the optim() procedure in R 2.10.1[2]. Standard errors were obtained by bootstrapping (N= 1000) from the data in Table S2.

Transition matrices and reproductive values

To quantify the effect of birth date on lifetime fitness in females, we derived matrix population models[1] for both skink populations. From the matrix models we calculated stage-specific reproductive values (RV's[1,3,4]) as a measure of stage-specific fitness. Since the model for lowland females (Fig S2) has 17 life history stages, the corresponding transition matrix is a 17×17 matrix. In block matrix notation,

$$\mathbf{M} = \begin{bmatrix} \mathbf{O}_1 & \mathbf{F} \\ \mathbf{G} & \mathbf{O}_2 \\ \mathbf{A}_1 \mid \mathbf{A}_2 & \mathbf{S} \end{bmatrix}. \quad \text{(A4)}$$

Here $\mathbf{O}_1$ and $\mathbf{O}_2$ are respectively 3×15 and 12×5 zero matrices. $\mathbf{G}$, $\mathbf{A}_1$ and $\mathbf{A}_2$ are, respectively, 12×12, 2×3 and 2×12 matrices corresponding to transitions from the non-breeding stages:

$$\mathbf{G} = (1-\mu)\,\mathrm{diag}[(1-\alpha_{\mathrm{E1}}),\cdots,(1-\alpha_{\mathrm{L4}})] \quad \text{(A5)}$$

$$\mathbf{A}_1 = (1-\mu)\begin{bmatrix} \alpha_{\mathrm{E1}} & \alpha_{\mathrm{M1}} & \alpha_{\mathrm{L1}} \\ 0 & 0 & 0 \end{bmatrix}, \quad \mathbf{A}_2 = (1-\mu)\begin{bmatrix} 0 & \cdots & 0 \\ \alpha_{\mathrm{E2}} & \cdots & \alpha_{\mathrm{L5}} \end{bmatrix}. \quad \text{(A6)}$$

Reproduction and survival of mature females is encoded by the 3×2 and 2×2 matrices

$$\mathbf{F} = \frac{1}{3}\begin{bmatrix} F_2 & F_{>2} \\ F_2 & F_{>2} \\ F_2 & F_{>2} \end{bmatrix}, \quad \mathbf{S} = \begin{bmatrix} 0 & 0 \\ 1-\mu & 1-\mu \end{bmatrix}. \quad \text{(A7)}$$

The stage-specific RV's are then given by the dominant left eigenvector $\mathbf{v}$ of $\mathbf{M}$:

$$\lambda \mathbf{v}^T = \mathbf{v}^T \mathbf{M}, \quad \text{(A8)}$$

where λ is the dominant eigenvalue of **M** and superscript T indicates matrix transposition. After plugging in the parameter estimates from Table S2, **v** was numerically calculated with the procedure eigen() in R 2.10.1.

For the highland population an identical procedure was followed, except that **M** was a 18×18 matrix.

Note that these RV calculations assume an even sex ratio and life history parameters that do not fluctuate between years. The results are shown in Table S3. For lowland females, early born individuals have an RV approximately 50% higher than late born individuals, while females born at intermediate birth dates have an RV closer to early born than to late born females. For the highland population, there was very little effect of birth date on RV; if anything, late born females had a slightly higher RV.

Individual-based simulations

Life cycle

Populations consisted initially of 5000 breeding females and 5000 breeding males, all 2 years old. Each year in a simulation, a mean annual temperature T_Y was drawn from a normal distribution with mean equal to the long-term mean temperature T_M and standard deviation σ_B, as estimated from climatic date (Table S2). Within a given year, each breeding female was assigned a temperature drawn from a normal distribution with mean T_Y and standard deviation σ_W (Table S2). If T_F was smaller than 0.43 σ_W below T_M, the female was classified as Early; if T_F was larger than 0.43 σ_W above T_M, the female was classified as Late; otherwise she was classified as interMediate. Thus, offspring had about a 1/3 chance of ending up in each birth date category. Each female was mated with a single random male (hence some males mated

multiply, while others remained unmated) and produced a clutch size from an age-specific distribution (Table S2). Juvenile survival was independent of temperature, sex and birth date (Uller, T. et al. submitted) and was scaled such that in the simulations the population as a whole remained approximately constant in size, with at most 5000 breeding females and 5000 breeding males.

Sex determination

Sex determination was modelled as a threshold polymorphism[5,6] (Fig. S3). This enabled us to take into account both the multi-factorial nature of sex determining processes[7-9] and allow for the evolution of genes of major effect, i.e., a single-factor genotypic sex-determining system[10]. Morphologically distinct sex chromosomes have not been described in snow skinks. However, because sex chromosomes are often cryptic in lizards[11,12], we set up our model to allow for genes of major effect according to both female (ZW/ZZ) and male (XY/XX) heterogamety systems. The sex of each offspring was determined by a combination of the alleles at 4 diploid gene loci and the maternal temperature T_F (Fig. S3) Males develop if levels of a particular gene product, produced by a single locus ('initial sex chromosomes'; see below), reach above a certain genetically determined threshold, θ ; females develop when the level of gene product fails to reach the threshold (Fig. S3). Two additional gene loci corresponded to the location (μ_c) and standard deviation (σ_c) of the curve determining the relative amount of gene product as a function of T_F (Fig. S3). The phenotypic values of μ_c, σ_c and θ were the averages of the two allelic values at the corresponding genes (i.e. additive gene action). In simulations where GSD was ancestral we set the threshold and curve loci so that the gene product produced by the Z (or X) chromosome initially caused offspring sex to develop according to karyotype.However, in every newborn offspring, each allele at the loci for μ_c, σ_c and θ had a 1% probability of mutating to a new allelic value equal to the old one plus a random number from a normal distribution with mean zero and standard deviation 0.1 (Table S2). Consequently, the sex determining system could evolve via changes in allele

values at the two loci describing the reaction norm, the threshold locus, and evolutionary loss of Z or W (or X or Y), which allows for both 'pure' GSD and TSD as well as mixed systems (i.e., when both variation at genetic loci and temperature variation influences sex within populations)[8]. We evaluated the results by examining the frequencies and distribution of alleles at the four loci and the sex ratios produced within and between years.

Simulation data analysis

Each parameter combination was coded in GNU g++ and run 20 times for 200,000 simulated years. Simulation data was statistically analyzed with R 2.10.1.

In order to quantify the degree to which sex determination depended on temperature in the simulations, a mutual information (*MI*) measure, a generalized measure of association based on entropy, was calculated according to (Schwanz & Proulx 2008)[13]. High *MI*-values correspond to strong association and a value of zero to complete independence. Specifically, *MI* is given by

$$MI = H_S + H_T - H_{TS} \tag{A9}$$

where H_S is the entropy associated with the sex ratio:

$$H_S = -S \log s - (1-S) \log(1-S) \tag{A10}$$

where S is the mean sex ratio (proportion sons) in the population, averaged over all breeding females. The entropy of the within-year (discretized) temperature distribution p_T is given by

$$H_T = -\sum_T p_T \log p_T. \tag{A11}$$

The joint entropy of temperature and sex ratio is given by

$$H_T = -\sum_T s_T p_T \log(s_T p_T) - \sum_T (1-s_T) p_T \log[(1-s_T) p_T] \ . \tag{A12}$$

For each simulation run, *MI* was averaged over the last 1000 generations. Confidence intervals for mean *MI* values averaged over multiple simulation runs (such as in Fig. S4) were obtained by bootstrapping.

Sensitivity analysis

The sensitivity of the model predictions to various assumptions and parameter values was analyzed by (1) varying the major-effect initial genetic sex determination system (ZW or XY); this had no qualitative effect on the model predictions. (2) By varying the amount of linkage (either recombination rate = 0 or 0.5) between the three loci for μ_c, σ_c and θ; again, this did not affect the qualitative model predictions. (3) By varying the difference in maturation rate ($\alpha_{Ej} - \alpha_{Lj}$) between early born and late born females from −75% to 75% of the estimated values, and by varying the between-year standard deviation σ_B in mean yearly temperature from −75% to +75% of the estimated values. The results are shown in Fig. S4 and clearly indicate that both population differences in the effect of birth date on maturation rate and population differences in climatic fluctuations are responsible for the evolutionary divergence in sex determination.

Evolution of alternative sex determining mechanisms

Our simulations using lowland parameter settings and GSD as the ancestral state consistently resulted in loss of sex chromosomes and lack of evolutionary branching of the reaction norm or threshold loci (Fig. 3; Fig. S5). Simulations using highland parameters had one of three outcomes. Firstly, sex chromosomes (according to ZW or XY systems) remained at the expected frequencies (1:3) that produced an average 50:50 sex ratio; neither of the other loci

contributed to sex determination (example in Fig. S6). Secondly, in the event of evolutionary loss or fixation of the W (Y) chromosome, disruptive selection on the threshold locus resulted in a novel genetic element with a major effect on sex determination, essentially generating a novel set of sex chromosomes (Fig. 3d, S5). Finally, in some relatively rare simulations, the initial sex chromosomes remained at some frequency in the population that deviated from 1:3, with a contributing minor effect on sex determination by one or several of the other loci (example in Fig. S6). Although our model assumes no fitness disadvantage of co-existence of genetic and environmental effects on sex determination, fitness reductions are likely when the heteromorphic chromosome has undergone substantial gene loss[14,15]. However, homomorphic sex chromosomes are common in reptiles[12], which facilitates developmental and, thus, phylogenetic changes in sex-determining systems[15,16].

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Table S1. Parameter values used in individual-based simulation model. Bold-faced values for measured parameters, italic values chosen independently of data, all others values were inferred.

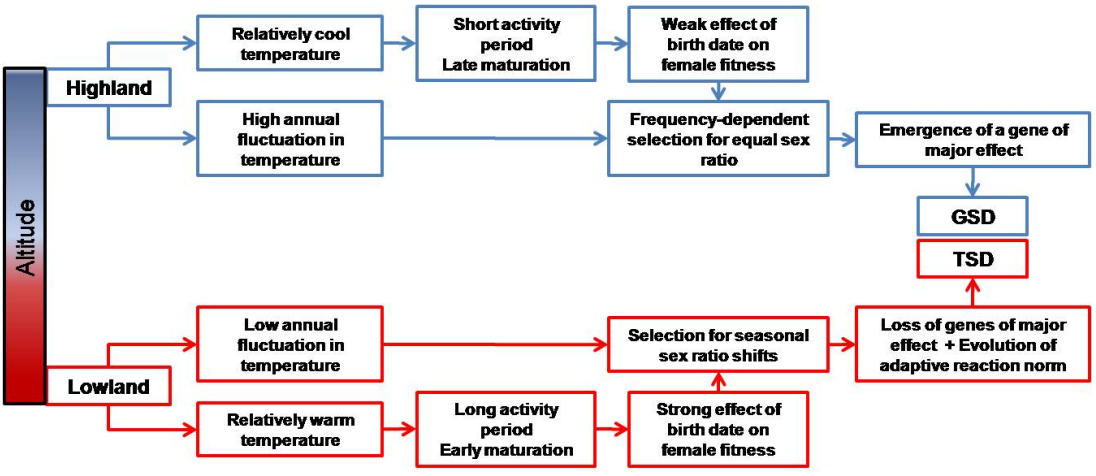
	Lowland population			**Highland population**		
Climate parameters						
Long term mean temp. T_M	**17.41** ºC			**15.43** ºC		
Std. dev. between years σ_B	**0.59** ºC			**1.45** ºC		
Std. dev. within years σ_W	**3.22** ºC			**4.27** ºC		
Life history parameters						
Adult mortality μ (/yr)	0.36			0.33		
Clutch size	Age = 2	Age > 2		Age = 2	Age = 3	Age > 3
1 eggs	0.458	0.138			0.048	0.026
2	0.417	0.496		0.875	0.349	0.174
3	0.125	0.294		0.125	0.422	0.331
4		0.073			0.157	0.297
5					0.024	0.126
6						0.038
7						0.009
Mean	F_2=1.67	$F_{>2}$=2.30		F_2=2.13	F_3=2.76	$F_{>3}$=3.47
Maturation rate α_{ij}	Early	Medium	Late	Early	Medium	Late
2 yrs old	0.291	0.217	0.167	0.056	0.028	0.021
3	0.724	0.764	0.312	0.468	0.702	0.543
4	1	0.658	0.563	0.626	0.787	0.841
5	1	1	1	1	1	1
Genetic parameters						
Mutation rate	*0.01*					
Std. dev. mutation size	*0.1*					

Table S2. Counts of females breeding for the first time at ages 2-5 years old, depending on birth date category (Early, interMediate, Late) and population.

	Lowland population			Highland population		
Age	**E**	**M**	**L**	**E**	**M**	**L**
2	7	13	5	4	3	1
3	8	23	5	21	49	17
4	2	3	4	10	11	8
5	0	1	2	4	2	1

Table S3. Estimated age and birth date-specific reproductive values (RV) of females from lowland and highland populations. RVs were normalized such that the oldest breeding females have unity RV.

Lowland population		Highland population	
Status	**RV**	**Status**	**RV**
Juvenile, early	0.181	Juvenile, early	0.194
Juvenile, medium	0.165	Juvenile, medium	0.220
Juvenile, late	0.122	Juvenile, late	0.209
Breeding, age 2	0.900	Breeding, age 2	0.818
Breeding, age > 2	1.000	Breeding, age 3	0.928
		Breeding, age > 3	1.000



spotted snow skink
(Niveoscincus ocellatus)

Fig. S1. Flow chart describing the climatic, physiological and evolutionary processes underlying population divergence in sex-determining systems in the snow skink, *Niveoscincus ocellatus*.

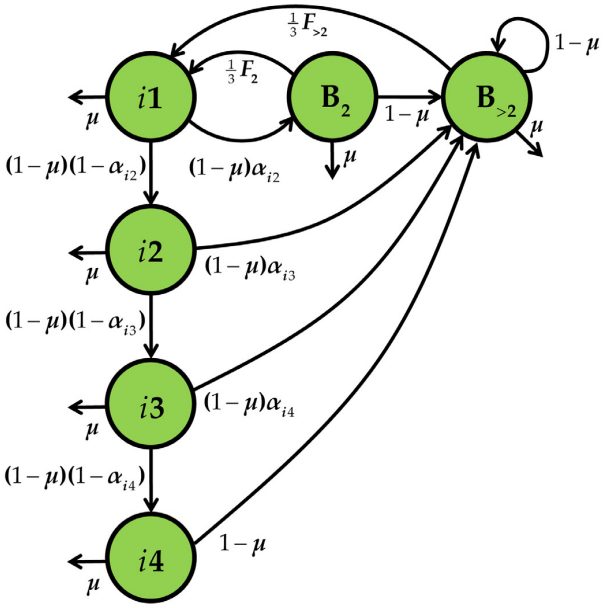


Fig. S2. Life cycle of lowland females in the simulation model. Circles indicate discrete life history stages, arrows transitions between them. B_2 and $B_{>2}$ are breeding females, aged 2 years or older, respectively. Non-breeding females aged 1-5 are in stages $i1$-$i5$, where i denotes the birth date category early, intermediate or late. All females have yearly mortality rate μ. Breeding females are uniformly (probability 1/3) distributed according to breeding/birth date and produce F_j daughters (j=2 or >2) that enter non-breeding age class 1. Non-breeders in birth date category i enter the breeding population at age j with probability α_{ij}, provided they survive with probability $1-\mu$.

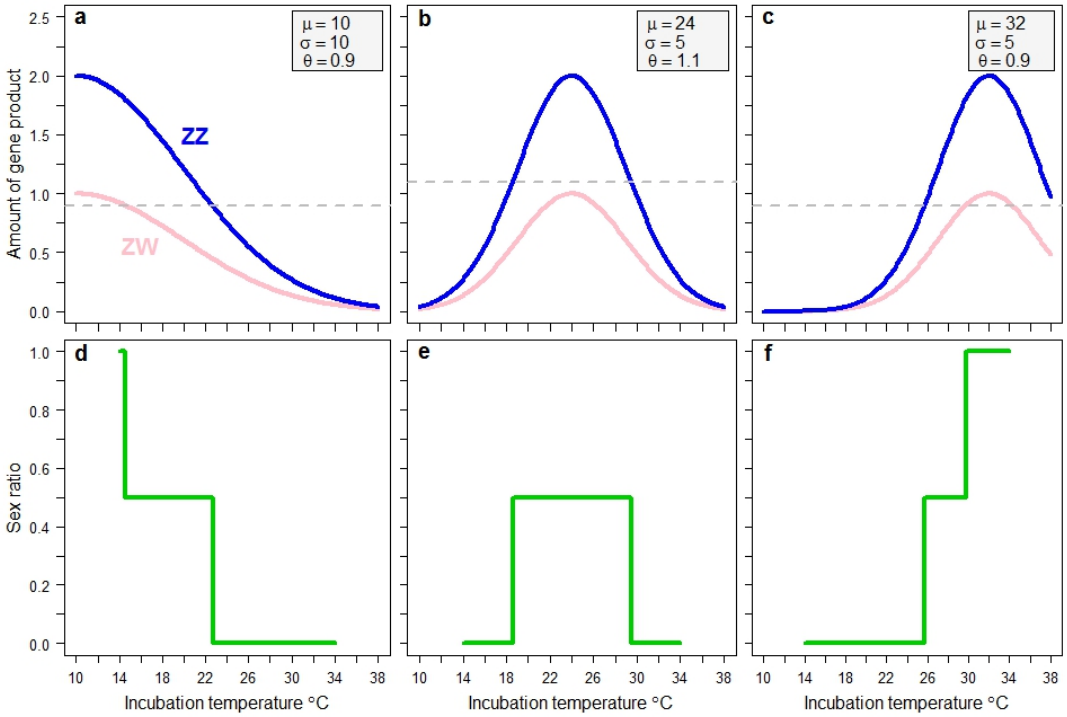


Fig. S3. Sex determination in the simulation model. The mechanism is a threshold system in which the amount of gene product (produced by the Z chromosome) determines offspring sex (panels **a-c**). If the amount is higher than the threshold, θ (dashed horizontal lines), males are produced and vice versa. This mechanism allows for a broad range of reaction norms of sex ratio as a function of temperature (examples in panels **d-f**).

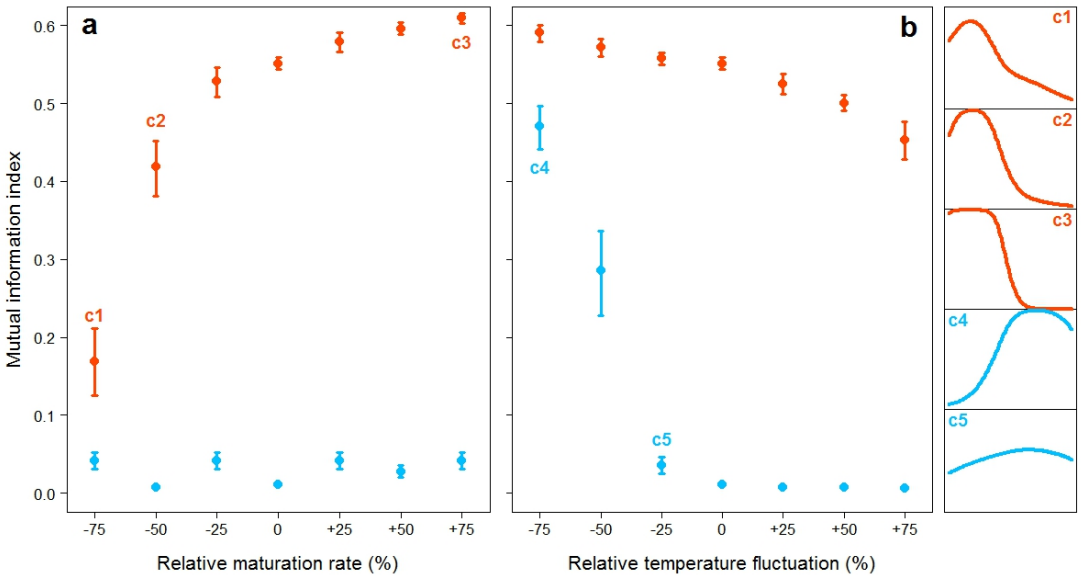


Fig. S4. Sensitivity analysis for how accurately temperature predicts cohort sex ratio (i.e., mutual information) (±SE). Lowland parameter estimates are given in red and highland in blue. **a**. Relative maturation rate for early and late born females. **b**. Relative annual fluctuation in temperature. Each parameter was varied between ± 25-75 % of its initial estimate based on field data from long-term studies. **c**. Evolved average reaction norms (cf. Fig. 3b/e) for a selection of parameter settings in panels a and b.

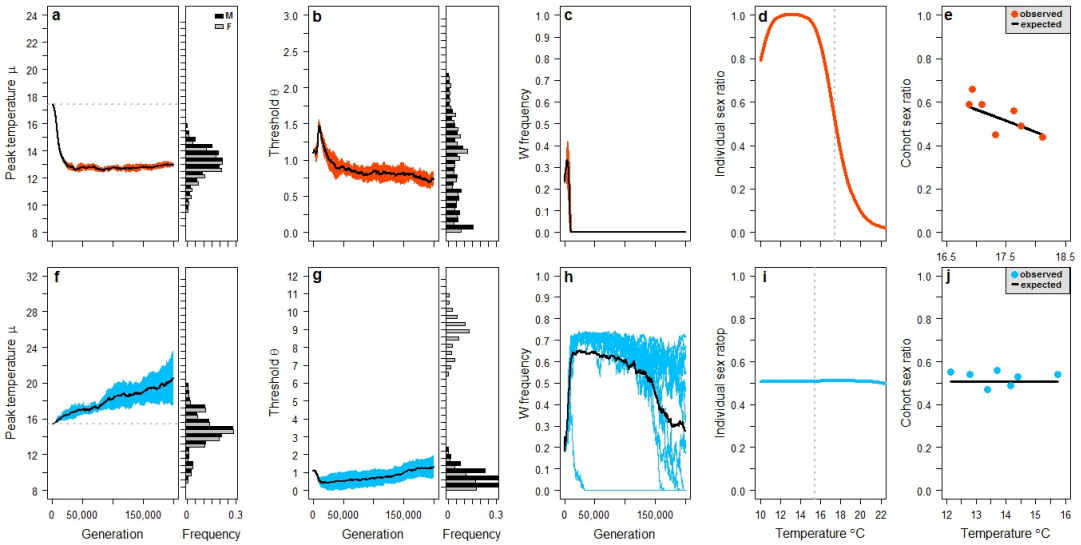


Figure S5. Summary of evolutionary simulation results. Upper panels show the result for simulations using lowland parameter settings and lower panels for simulations using highland parameter settings. **a/f.** Average allele values (± SD between runs) as a function of time and final distribution of alleles (a single run) for the peak temperature locus; **b/g.** Average allele values (± SD between runs) as a function of time and final distribution of alleles (a single run) for the threshold locus (from a run where W is lost). Note disruptive selection for the highland parameter settings; **c/h.** Frequency of the W chromosome at the sex chromosome locus as a function of time; black line: average over runs; blue lines individual runs. **d/i.** Evolved average reaction norm for offspring sex ratio as a function of maximum mean temperature experienced during first half of gestation. Vertical dotted lined is the average temperature experienced by natural populations; **e/j.** Predicted (from model; solid line) and observed (from natural populations; filled circles) cohort sex ratios for annual mean maximum temperature in the wild.

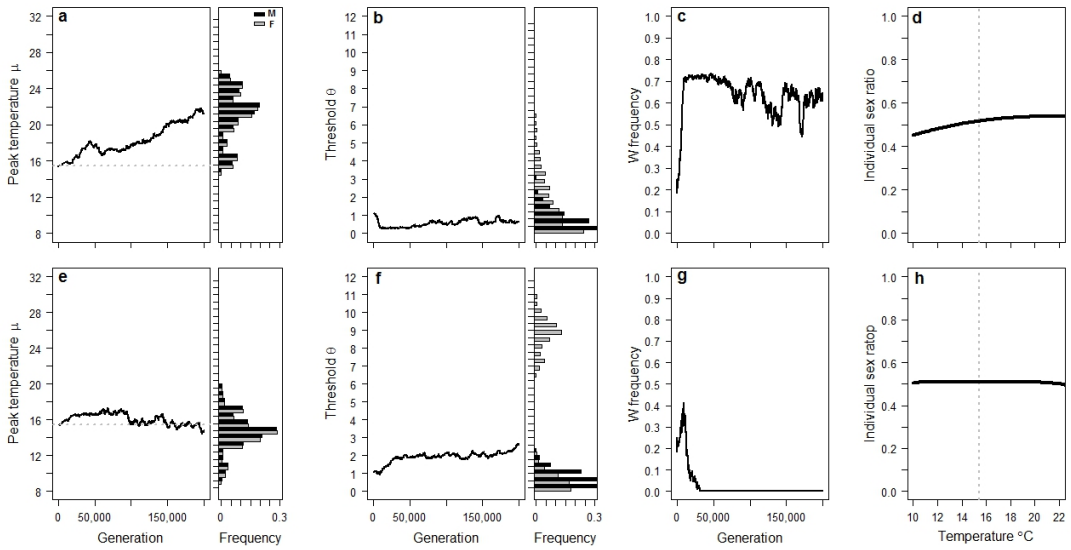


Fig. S6. Two single simulation runs parameterized with the same highland population data (Table S1) and identical initial conditions, yet with very different evolutionary outcomes for sex determination. In the top panels, the population starts with female heterogamety (females ZW, males ZZ) and rapidly evolves towards a sex determining system with male heterogamety (females WW, males ZW). The frequency of the W-chromosome evolves from 25% to about 70% (c), while the threshold alleles evolve towards a skewed distribution close to zero with little sex-specific differentiation. In contrast, in the bottom panels, the same initial state of female heterogamety evolves towards a new system with female heterogamety. The W-chromosome rapidly goes extinct (g), while disruptive selection at the threshold locus (f) creates a de facto novel genetic sex determining locus where males are homozygous for alleles coding for low thresholds and females are heterozygous for low-threshold and high-threshold alleles. In both scenarios the sex ratio remains close to 50:50, regardless of temperature (d and h).