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Interplay of Genotypic and Thermal Effects on Sex Determination Shapes Climatic Distribution in Herpetofauna

Edina Nemesházi[1] | Veronika Bókony[2]

[1]Konrad Lorenz Institute of Ethology, Department of Interdisciplinary Life Sciences, University of Veterinary Medicine Vienna, Vienna, Austria | [2]Department of Evolutionary Ecology, Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary

Correspondence: Edina Nemesházi (nemeshazi.edina@protonmail.com)

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ABSTRACT

Aim: Sex is a fundamental trait of many organisms, and sex-determination systems are diverse across the tree of life. Environmental temperature can influence sexual development not only in temperature-dependent sex-determination systems, but also in species with genotypic sex determination (GSD). Temperature-induced sex reversal, resulting from combined effects of GSD and environmental temperatures, may greatly affect population dynamics and extinction risk. However, the scarcity of research on sex reversal has constrained the assessment of its evolutionary-ecological significance. We assessed if climatic distribution differs between female-heterogametic (ZW/ZZ) and male-heterogametic (XX/XY) species in herpetofauna when taking into account thermal reaction norms (TRN; i.e., how temperature affects progeny sex ratio), as suggested by the 'asymmetrical sex reversal' theory.
Location: Global.
Time Period: 1970–2000.
Major Taxa Studied: Amphibia, Reptilia.
Methods: For all extant species where information was available, we collected data on their types of heterogamety and TRN from the HerpSexDet database, and on various aspects of temperature across their native distribution range from the WorldClim database. We used phylogenetic generalised least squares to compare environmental temperatures between XX/XY and ZZ/ZW systems within each type of TRN.
Results: Climatic distribution of extant species is explained by the combination of their sex-chromosome system and the shape of their TRN across amphibians and in infraorder Gekkota among reptiles. In these taxa, directions of the climatic differences conform to the 'asymmetrical sex reversal' theory.
Main Conclusions: Our results underscore the importance of temperature-induced sex reversal in phylogeography, evolution, and species conservation under climate change, and highlight the need for more empirical research on sex reversal in nature.

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1 | Introduction

The great diversity of sex-determination systems across animal phylogenies has long been puzzling researchers. While mammals and birds possess genotypic sex determination (GSD), where individuals develop gonads corresponding to their sex chromosomes, the picture is much more complex in ectothermic vertebrates, where besides GSD, environmental sex determination (ESD) is also widespread (Bachtrog et al. 2014). In ESD systems, factors such as thermal and social environmental stimuli determine the individual's sexual phenotype, and the most common ESD is temperature-dependent sex determination (TSD; Ashman et al. 2014; Bachtrog et al. 2014; Charnov and Bull 1977; Nemesházi and Bókony 2023). The type of sex determination has crucial implications for a diverse array of biological phenomena, including genome evolution, adaptive radiation, and the evolution of life-history and demographic traits and reproductive systems (Cayuela et al. 2021; Lemaître et al. 2020; Organ et al. 2009; Pipoly et al. 2015, 2023; Sabath et al. 2016; Sultanova et al. 2023; Valenzuela and Adams 2011; Xirocostas et al. 2020). Climatic change is inferred to be a major driver of the evolution of sex-determination systems (Cornejo-Páramo, Dissanayake, et al. 2020; Valenzuela and Adams 2011), so understanding the mechanisms and consequences of these evolutionary processes is critical in light of contemporary rapid climatic changes.

Traditionally, GSD and ESD were viewed as mutually exclusive dichotomic categories. However, a growing body of evidence shows that we need to move beyond this simplifying view for a full understanding of sex-determination systems (Holleley et al. 2016; Nemesházi and Bókony 2022, 2023; Roush and Rhen 2018; Sarre et al. 2004). Numerous experiments demonstrated that environmental factors can override the sexual development otherwise determined by sex chromosomes (and/or other genomic elements) in GSD, leading to sex reversal: a mismatch between genotypic sex and phenotypic sex (Baroiller and D'Cotta 2001; Geffroy and Douhard 2019; Nemesházi and Bókony 2022, 2023). Sex-reversed individuals are difficult to detect in nature because identifying genotypic sex is challenging in non-model species. Nevertheless, when researchers had the genotypic markers to look for sex reversal in free-living populations of fish, amphibians, and reptile species, they usually did find it occurring with considerable frequency (Alho et al. 2010; Baroiller and D'Cotta 2016; Castelli et al. 2021; Dissanayake, Holleley, and Georges 2021; Dissanayake, Holleley, Deakin, et al. 2021; Geffroy and Wedekind 2020; Lambert et al. 2019; Nemesházi et al. 2020). These reports included six amphibian and two reptile species (Nemesházi and Bókony 2023), out of which temperature-induced sex reversal was also demonstrated under experimental conditions in the central bearded dragon (*Pogona vitticeps*; Castelli et al. 2021; Holleley et al. 2015), the alpine three-lined skink (*Bassiana duppereyi* = *Acritoscincus duperreyi*; Radder et al. 2008; Shine et al. 2002), Dybowski's frog (*Rana dybowskii*; Xu et al. 2021) and the agile frog (*Rana dalmatina*; Mikó et al. 2021; Ujszegi et al. 2022). The cause of sex reversal in free-living animals is never certain because several kinds of environmental stimuli may cause sex reversal, including pollutant chemicals and pathogenic infections (Baroiller and D'Cotta 2001, 2016; Geffroy and Douhard 2019; Nemesházi and Bókony 2022; Ujszegi et al. 2024). Nevertheless, temperature is one of the main suspects, as sex determination often has a thermal reaction norm (TRN) in ectothermic vertebrates (Geffroy et al. 2021; Nemesházi and Bókony 2023; Ospina-Álvarez and Piferrer 2008). The TRN describes the way in which developmental temperatures influence the phenotypic sex ratios or sex-reversal rates of the offspring. For example, *P. vitticeps* incubated at 26°C–32°C exhibits equal sex ratios, but higher temperatures trigger male-to-female sex reversal (i.e., genotypic males develop into phenotypic females) that results in almost complete female bias at 36°C (Holleley et al. 2015). Because offspring sex ratios are more female-biased at higher temperatures, this species exhibits an MF pattern of TRN. The same type of TRN is combined with sex reversal in the opposite direction in *A. duperreyi*, where low temperatures induce female-to-male sex reversal (Radder et al. 2008; Shine et al. 2002). The opposite pattern of TRN, i.e., FM, occurs in *Rana* species due to female-to-male sex reversal at high temperatures (Mikó et al. 2021; Ujszegi et al. 2022; Xu et al. 2021). TRN patterns were found in many more GSD species by investigating the phenotypic sex ratios of offspring (Nemesházi and Bókony 2023), suggesting that mixed sex-determination systems characterised by thermal reversal of otherwise genotypically determined sex may occur in many more taxa than currently recognised.

Theoretical studies caution that sex reversal can have wide-ranging consequences for population dynamics, microevolution, and extinction risk, because sex reversal may lead to sex-ratio bias, which may then influence the fitness of sex-reversed and sex-concordant individuals differently (sometimes in favour of the sex-reversed) (Bókony et al. 2017; Grossen et al. 2011; Nemesházi et al. 2021; Schwanz et al. 2013, 2020). Furthermore, empirical data show that sex reversal can influence various fitness-related traits including physiology, life history, and behaviour (Bókony et al. 2021, 2025; Jones et al. 2020; Li et al. 2016; Wild et al. 2023). Thus, temperature-induced sex reversal may have both evolutionary significance and implications that could help identify vulnerable hotspots for biodiversity conservation under the concurrent climate change. However, due to the scarcity of empirical data on sex reversal (see Figure 1 for herpetofauna, and for other taxa see Ning et al. 2019; Ospina-Álvarez and Piferrer 2008; Parvathy et al. 2021), it remains a challenge to evaluate its role in the evolution of ecological and other species-level traits across the tree of life.

As a result of sex-chromosome evolution, the type of GSD system might influence the direction in which sex reversal could occur in a species. According to Muller's ratchet model, deleterious mutations tend to accumulate on the hemizygous sex chromosome which always occurs in heterogametic form (i.e., the Y chromosome in XX/XY and the W chromosome in ZW/ZZ systems; Muller 1964). If heterogametic individuals undergo sex reversal, 25% of their offspring will possess this chromosome in a homogametic form (i.e., YY or WW genotypes resulting from mating of XY males and XY females or ZW males and ZW females, respectively). Production of this new, potentially infertile or lethal genotype is expected to reduce fitness in sex-reversed heterogametic individuals. By contrast, sex reversal may be neutral in the homogametic sex, or might

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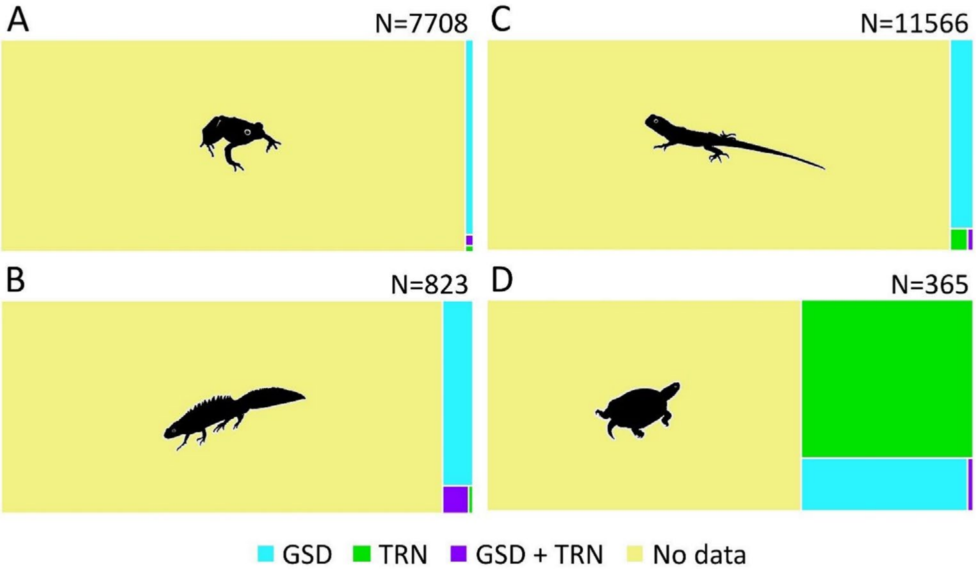

FIGURE 1 | Availability of data on sex determination in the major taxonomic orders of amphibians and reptiles. Taxonomic orders of Anura (A), Caudata (B), Squamata (C) and Testudines (D) are shown. The number at the top-right corner of each panel indicates the number of extant species (Frost 2024; Uetz et al. 2024). Proportions are calculated based on the number of species with data on the type of heterogamety (GSD), thermal effects on sex determination (TRN) or both (GSD + TRN), as collected in the HerpSexDet database (Nemesházi and Bókony 2023). Orders where no species are known to have GSD (Crocodilia, Rhynchocephalia) or no data on sex determination is available (Gymnophiona) are not included in the figure.

even be advantageous by producing the phenotypic sex and/or progeny sex ratio that has the highest pay-off in the given environment (Bókony et al. 2025; Geffroy and Douhard 2019; Holleley et al. 2015; Nemesházi et al. 2021). Furthermore, sex-antagonistic genes (van Doorn and Kirkpatrick 2010) accumulating on the Y or W chromosome may also cause disadvantage in sex-reversed XY or ZW individuals. Therefore, natural selection should favour resistance to sex reversal in the heterogametic sex, leading to biased sex-reversal patterns where sex-reversing environmental conditions affect the homogametic sex more prominently (Nemesházi and Bókony 2022; Schwanz et al. 2013). With other words, female-to-male sex reversal should be more easily induced in genetic females (XX) in XX/XY systems, while in ZW/ZZ systems, genetic males (ZZ) should be more likely to undergo sex reversal and become phenotypic females, leading to 'asymmetrical sex reversal' (Bókony et al. 2017; Bull 1981; Nemesházi and Bókony 2022; Schwanz et al. 2013); a theoretical explanation of the empirical pattern described as 'Witschi's rule' (Witschi et al. 1958). This hypothesis is supported in herpetofauna by the scarcely available empirical data from laboratory experiments (reviewed in Nemesházi and Bókony 2022) and also from field observations, i.e., sex-reversed individuals found in nature were typically either ZZ females (Castelli et al. 2021; Holleley et al. 2015) or XX males (Alho et al. 2010; Dissanayake, Holleley, and Georges 2021; Dissanayake, Holleley, Deakin, et al. 2021; Lambert et al. 2019; Nemesházi et al. 2020; Xu et al. 2021).

The theory of asymmetrical sex reversal provides testable predictions for the interplay between genotypic and thermal effects on sex determination (i.e., thermal sex reversal) under different climatic conditions (Figure 2). Accordingly, when directional climate change results in a thermal environment which would induce sex reversal in heterogametic individuals, sex-reversal resistance is expected to evolve in response to fitness reduction due to the production of YY or WW offspring, and this resistance should then allow the prevailing sex-chromosome system to persist longer (Figure 2). By contrast, at temperature ranges causing sex reversal in the homogametic sex with high frequency, sex-reversed individuals produce the rarer sex in excess (i.e., all-XX or all-ZZ progeny), and thus gain immediate fitness advantage. Consequently, the sex-chromosome system should gradually disappear (Figure 2) either by transitioning into another sex-determination system or by population extinction (Bull 1981; Grossen et al. 2011; Nemesházi et al. 2021; Schwanz et al. 2013). Therefore, the climatic distribution of extant species should be constrained by the combination of their sex-chromosome system and their TRN. The latter varies from FM pattern (low and high temperatures producing females and males, respectively) to the opposite MF pattern through the FMF pattern (where males are produced at intermediate temperatures and females at the extremes). Asymmetrical sex reversal predicts, as detailed in Figure 2, that under FM TRN pattern, ZW/ZZ systems should be found in warmer environments than XX/XY systems, and the opposite difference is expected under MF pattern. For the FMF TRN pattern, where temperature has a non-monotonous effect on sex and thus on the expected outcomes, whether and how the two GSD systems should differ in climatic distribution is predicted to depend on the extent of directional climate change each species has experienced (Figure 2). The logic of these predictions may be easiest to understand in the example of FM TRN pattern combined with endless habitat warming: this combination causes female-to-male sex reversal with ever-increasing frequency. In this scenario, ZW/ZZ systems are expected to be stabilised by sex-reversal resistance of the ZW genotype if deleterious mutations are accumulated on W. In contrast, in an XX/XY system, sex-reversed XX males will produce only XX offspring, benefitting from female-biased

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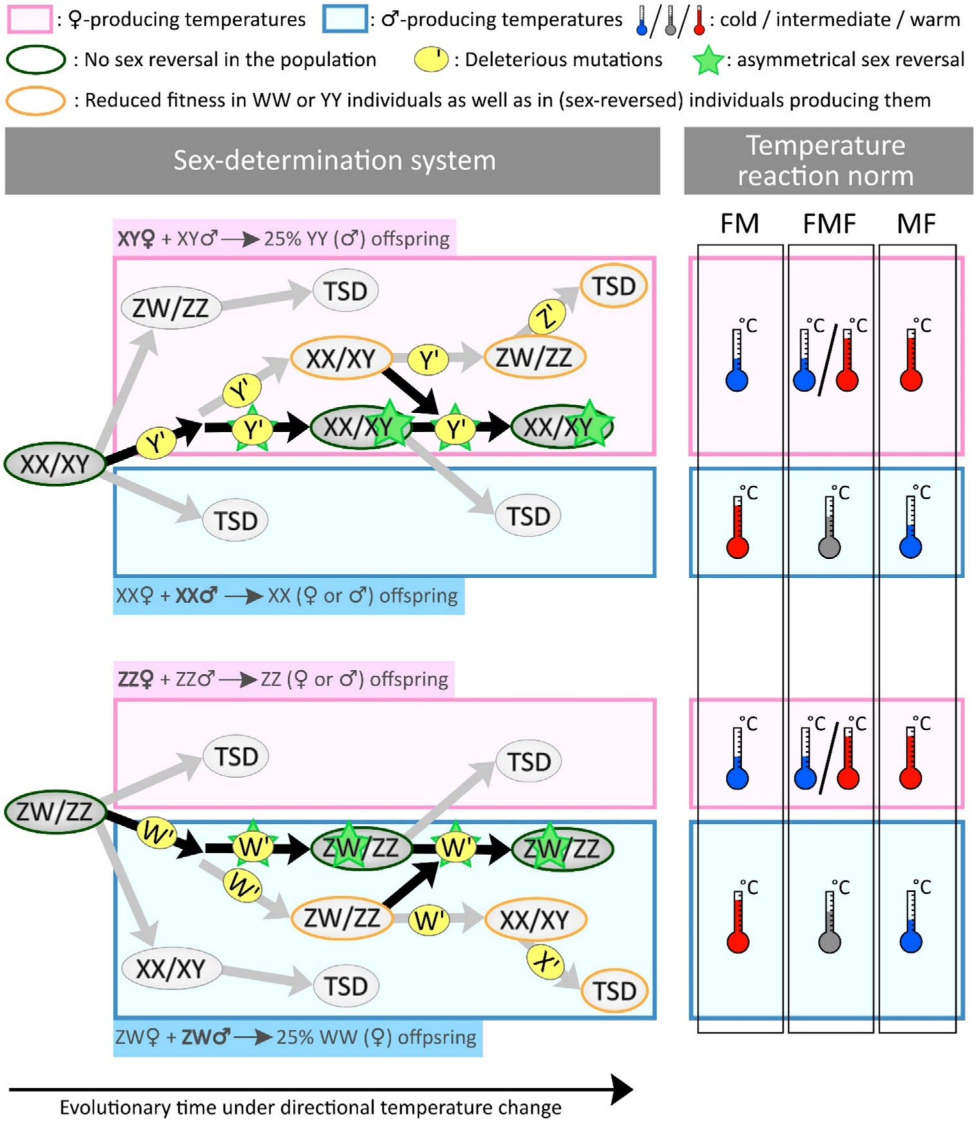

FIGURE 2 | Hypothesized evolutionary pathways of sex-determination systems with thermal sex reversal. Directional climate change results in environmental temperatures that trigger male-to-female or female-to-male sex reversal (pink and light blue rectangles, respectively) depending on the pattern of thermal reaction norm (TRN) of the species. Changes in sex-chromosome frequencies cause turnovers between GSD systems, that become TSD when one sex chromosome disappears from the population. The paths denoted by grey arrows represent evolutionary scenarios predicted by earlier models (sex-ratio selection is key; Bull 1981; Grossen et al. 2011; Nemesházi et al. 2021). In systems denoted by orange circles, the population suffers increasing fitness reduction, and turnovers between these systems are only possible if the YY or WW genotype is viable and at least somewhat fertile; otherwise, early extinction is expected (Nemesházi et al. 2021). The paths denoted by black arrows illustrate how asymmetrical sex reversal (Nemesházi and Bókony 2022) may stabilise one heterogametic system, depending on the experienced environmental temperatures (symbolised by the thermometer icons on the right) and TRN. Although these evolutionary pathways may be altered by evolution of new TRNs (Quinn et al. 2011; Schwanz et al. 2013), new sex-determination genes and new sex chromosomes (Abbott et al. 2017; Grossen et al. 2011; Jeffries et al. 2018; Kratochvíl et al. 2021), for simplicity the illustrated scenarios assume no such changes.

offspring sex ratio in a male-biased population. Production of XX-only offspring will lead to the loss of Y, and consequently the end of the XX/XY system. Therefore, under FM pattern, XX/XY systems may only persist in relatively colder habitats, while in warmer habitats ZW/ZZ systems should more likely occur (Figure 2). For FMF TRN pattern, predictions of climatic differences between GSD types depend on the extent of directional climate change. As long as these species experience moderate temperature increase, the climatic distribution of different GSD types may correspond with predictions for the FM pattern, but it should increasingly resemble the opposite MF pattern as warming progresses in their habitats.

In this study, we used an indirect macroevolutionary approach to address the potential evolutionary importance of thermal sex reversal in herpetofauna by assessing whether the geographical distribution of contemporary GSD species conforms to the predictions of asymmetrical sex reversal. Our approach presumed that any ectotherm GSD species can theoretically undergo sex reversal by high or low temperatures, provided

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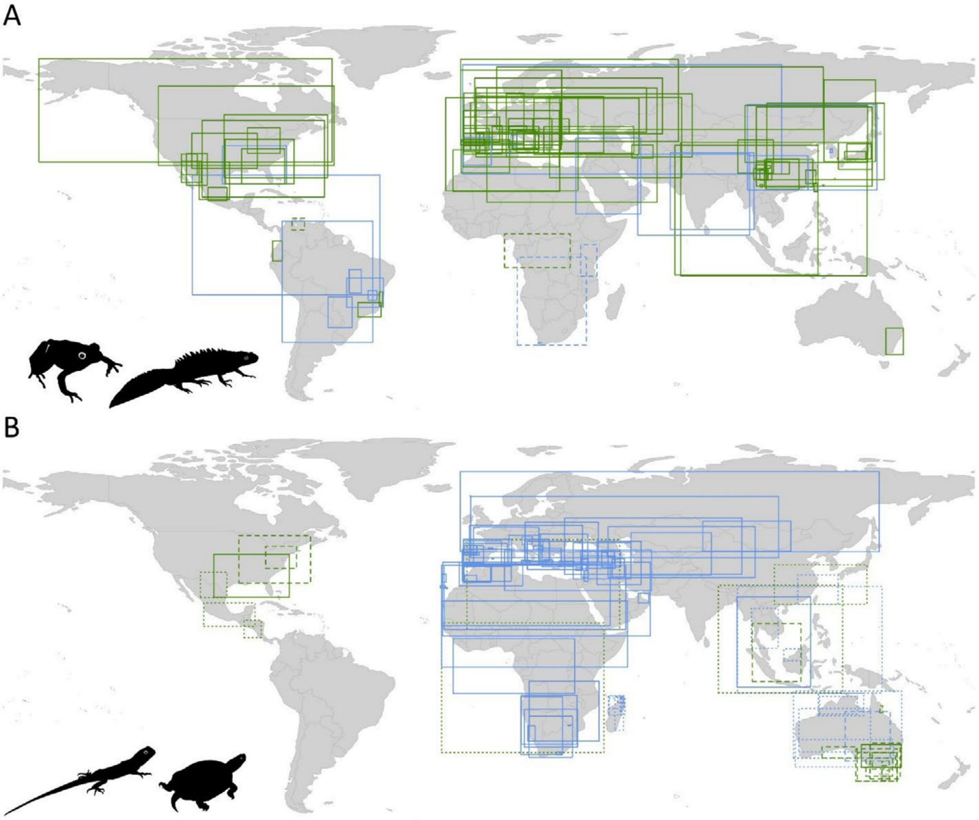

FIGURE 3 | Global distribution of amphibian (A) and reptile (B) species included in the analyses. Rectangles represent the extent of the native distribution range of each species, calculated as described in the Methods section. Rectangle colours denote GSD type (green: XX/XY and blue: ZW/ZZ), while the border types denote TRN patterns (solid line: FM, dashed line: MF, dotted line: FMF). Note that the distribution ranges of three reptile species (*Emoia nigra*, *Hemidactylus frenatus*, *Lepidodactylus lugubris*) are not shown, because they occur (at least in part) on islands across the Pacific Ocean; therefore, the extent of their distribution range could not be denoted by one rectangle on the conventionally centred world map.

that those are not lethal for the species (Quinn et al. 2011). We tested the predictions of asymmetrical sex reversal utilising HerpSexDet, the most complete dataset of GSD types and TRN patterns across amphibians and reptiles (Nemesházi and Bókony 2023), combining it with 16 variables calculated from temperature data of the WorldClim database (Fick and Hijmans 2017) to describe the climate (thermal environment) of each species' entire geographical distribution (Figure 3). First, we used phylogenetic generalised least-squares (PGLS) models to analyse how each climatic variable is related to GSD type within each TRN pattern. Because TRN has rarely been studied in GSD species, we assumed that species where no such information is available would possess a TRN similar to their close relatives. This assumption is based on the facts that only one TRN type was reported in more than half of the taxonomic families with multiple TRN records in herpetofauna (Nemesházi and Bókony 2023), and in families with more than one TRN type, there is often a majority type that most species share (Katona et al. 2021; Krueger and Janzen 2023; Nemesházi and Bókony 2023). Second, we collected data on the temperatures likely experienced over the species' geographical range during the period of sex determination. This was necessary because the sensitive window of sexual development, when environmental conditions may cause sex reversal, occurs during early ontogeny, but the timing of this period in nature is not known for many species. Thus, we identified the climatic variables (out of the 16) that correlate most strongly with the temperature of larval or embryonic development in each taxonomic class, using a smaller subset of amphibian and reptile species. We used this information to pinpoint the PGLS models that provide the most relevant test of our predictions.

2 | Methods

2.1 | Sex Determination

We collected species-specific data on the type of GSD and TRN of sexual development from the HerpSexDet database version 1.1, the most up-to-date collection of literature data on sex determination across amphibian and reptile species (Nemesházi and Bókony 2023). For the sake of the analyses, we classified both simple (i.e., one sex chromosome pair) and complex (i.e., multiple sex chromosomes) male-heterogametic systems as 'XX/XY', and simple and complex female-heterogametic systems as 'ZW/ZZ'. We excluded four species with rare GSD types (i.e., W0/00 and ZWY systems, and species that have both ZW/ZZ and XX/XY populations) for which our predictions are not straightforward to apply. Because TRN data were not available for most GSD species, we assigned one of the three common TRNs (i.e., FM, FMF or MF) to each taxonomic family that we included in the analyses. All families without any information on TRN pattern were excluded. The basis of our categorisation was that the type of sex determination is relatively well-conserved

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within taxonomic families of vertebrates (Katona et al. 2021; Krueger and Janzen 2023; Nemesházi and Bókony 2023; Pipoly et al. 2015). In principle, we included families where one TRN pattern was reported for at least two species (regardless of GSD being identified in them or not) and no other TRN pattern was reported. When more than one TRN pattern occurred in a family, we aimed to find phylogenetic clades below the family level that conformed to the above criteria (according to the Open Tree of Life Synthetic Tree; OpenTreeOfLife et al. 2019). Additionally, we considered families where TRN pattern was available for only one species, but we only included such families in the analyses if that TRN pattern was supported by TRN data in the phylogenetically closest family. Thus, we assigned the species-specific TRN to each species where it was available, and the TRN characteristic to its clade to each species with unknown TRN (see detailed justification in Table S3). Out of the 8 amphibian and 14 reptile families for which both GSD and TRN data were available, we included species from a total of 7 amphibian and 7 reptile families in our analyses by the above criteria (for detailed species-specific data, consult the 'TSD_type' column in the HerpSexDet database that lists TRN patterns regardless of the presence or absence of sex chromosomes). While numerical parameters of TRN (i.e., temperature thresholds) can differ between and even within species, here we categorised clades by the overall shape of TRN, which to our knowledge does not vary between individuals of the same species.

2.2 | Spatial Distribution

We collected data on the species' distribution ranges, preferably as spatial polygons obtained from the IUCN Red List of Threatened Species (spatial database version 2022-2 (IUCN 2023); hereafter IUCN). Some species were not present in the IUCN datasets or we judged that the available data were insufficient for further processing: the polygons were too small for masking the raster grid cells (see Section 2.3 below), or the coordinates assigned to the polygons were noticed to be inaccurate. In these cases, if available, spatial occurrence data were obtained from the database of the Global Biodiversity Information Facility (hereafter GBIF; GBIF 2022). We focused on the native distribution range where the species are currently extant and not introduced. Specifically, IUCN spatial polygons categorised as 'introduced', 'assisted colonisation' or 'vagrant' were excluded and the seasonality was required to be set either as 'resident' or 'breeding season'; and only GBIF datapoints categorised as 'present' with coordinate uncertainties below 10 km were included. Because GBIF provides no information on whether a population is introduced or not, we checked for introduced populations of the concerned reptile species in The Reptile Database (Uetz et al. 2022) (we did not find such information for the concerned amphibian species elsewhere). As we did not find indication of introduced populations of the species with GBIF data, we assumed that all datapoints represented native occurrences. We excluded those species from further analyses that had less than 10 GBIF datapoints after this filtering. All in all, spatial distribution data were collected from IUCN for 93 amphibian and 107 reptile species, and from GBIF for 2 amphibian and 6 reptile species. Geographical occurrence shapes were defined either as polygons provided by IUCN, or as GBIF datapoints converted to shape files using the 'writeOGR' function of the 'rgdal' package (Bivand et al. 2022) in R 4.2.1 (R Core Team 2022). For visualisation purposes on Figure 3, maps were drawn by the 'mapCountryData' function of the 'rworldmap' R package, and rectangles were added by the 'rect' function in R 4.3.2 (R Core Team 2023) for each taxonomic class; these final maps were subsequently arranged on the figure panels in INKSCAPE 1.2 (https://inkscape.org).

2.3 | Climatic Variables

Data calculations from spatial datasets were performed in R 4.2.1 (R Core Team 2022). We obtained temperature data in 5-min spatial resolution from the WorldClim database version 2.1 (Fick and Hijmans 2017) across a 30-year period (1970–2000, the time span for which historical WorldClim data are available). Out of the available 19 'bioclimatic' variables, we used those that we deemed likely to encompass temperature data relevant to the offspring-development season of herpetofauna: annual mean temperature (BIO1), maximum temperature of the warmest month (BIO5), mean temperature of the wettest quarter (BIO8), and mean temperature of the warmest quarter (BIO10). We cut out the geographical occurrence shapes from each raster band using the 'mask' function ('raster' package; Hijmans 2022). From these cuts, we obtained the temperature values with the 'getValues' function ('raster' package; Hijmans 2022) and subsequently calculated the mean, minimum, maximum, and median values of each of the four WorldClim rasters (hereafter these 16 variables will be referred to as WorldClim variables) across each species' native distribution range. For visualisation purposes, the westernmost, easternmost, southernmost, and northernmost coordinates of the distribution were calculated by the 'extent' function of the 'raster' package (Hijmans 2022) for polygons downloaded from the IUCN database; while for GBIF datapoints, they were calculated as the minimum and maximum values of the X and Y coordinates across datapoints, respectively (based on these calculations, spatial distribution of the studied species is shown in Figure 3).

The period when sexual development is sensitive to environmental temperatures (referred to as 'sex-determining period' henceforth) occurs during early ontogeny, during the larval phase in amphibians (Chardard et al. 2004; Gramapurohit and Phuge 2015; Mikó et al. 2021) and the embryonic phase in reptiles (Ewert et al. 1994; Holleley et al. 2015; Radder et al. 2008). Temperature during this period would be the most relevant to our study; however, the timing of this period is unknown for many species. Therefore, we collected information from the literature on the timing of the larval/embryonic season (in months) for a subset of the analysed species, aiming to identify the months that could best represent this season across multiple populations and regions (see columns 'Early_embryonic_development' and 'Breeding_note' in the data table: see Data Availability Statement). While we acknowledge that between-individual variations may also exist in the timing of breeding, these variations are expected to be much smaller than the variations between species. To approximate the sex-determining period, we estimated the timing of the larval/embryonic period in two ways. First, we took the month(s) when mating occurs in species with external fertilisation, and the month(s) when egg-laying occurs in oviparous species. For viviparous species (where sex determination occurs before birth), we took the month(s) when fertilisation occurs or gravid females appear, or

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the mating season if the previous two were not reported. Second, we extended the period described in the previous two sentences by 1 month because the length of the larval/embryonic developmental phase, the timing of the temperature-sensitive window within that phase, and the developmental stage of embryos at egg laying can differ between species. For both approximations of the sex-determining period (i.e., the non-extended and the extended larval/embryonic periods), we used monthly average temperature data in 5-min resolution from the WorldClim database version 2.1 (Fick and Hijmans 2017) to calculate mean, median, minimum and maximum temperatures across the native range (hereafter SexClim variables) for each month of the sex-determining period, by the same computational methods as described above for the WorldClim variables. Subsequently, we calculated the mean and median temperatures for the entire sex-determining period (i.e., mean of the monthly mean temperatures across the native range, and median of the monthly median temperatures across the native range, respectively) as well as for the coldest and the warmest month within that period. Furthermore, we assessed potential temperature extremes for larval/embryonic development by calculating the average temperatures of the coldest and warmest parts of the natural distribution range during the coldest and warmest month, respectively. In those species where the embryonic/larval season reportedly differed along a South–North cline (10 amphibian species; see column 'Early_embryonic_development' in the data table: see Data Availability Statement), we divided the natural distribution range into maximum of three subranges at equal distances along its latitudinal extent using the 'gIntersection' function ('rgeos' package; Bivand and Rundel 2022), and calculated the related SexClim variables separately for each subrange. Then, we calculated the relevant statistics (mean, median, minimum or maximum, depending on the variable) from these subranges and assigned a single value for each SexClim variable for each concerned species.

We assumed that the WorldClim data adequately reflect the relevant climatic differences not only among reptiles (whose offspring typically develop in terrestrial nests) but also among amphibian species. Although many amphibian species have aquatic larvae, those almost exclusively occupy shallow freshwater bodies, where water temperatures correlate well with air temperatures (Livingstone and Lotter 1998; Markovic et al. 2013). Note that, due to lack of TRN information, our dataset does not include the cave-breeding amphibian genus *Hydromantes* (Lunghi et al. 2018) nor viviparous sea snakes, where the land-surface temperatures of the WorldClim database might not be informative for conditions experienced by the offspring. Our final dataset, for which we obtained WorldClim variables, consisted of 95 amphibian and 113 reptile species with either male or female heterogamety as well as either species- or clade-specific information on the TRN of sexual development. Out of these, we found SexClim data for 73 amphibian and 47 reptile species. The final dataset is available on Figshare (see Data Availability Statement).

2.4 | Statistical Analyses

All statistical analyses were run in R 4.3.2 (R Core Team 2023). We built phylogenetic generalised least squares (PGLS) models (Freckleton et al. 2002) using the 'gls' function of the 'nlme' package (Pinheiro et al. 2023). To control for the phylogenetic relatedness among taxa, we used the Open Tree of Life Synthetic Tree (OpenTreeOfLife et al. 2019) that we accessed using the 'rotl' package (Michonneau et al. 2016). The resulting topology is available on Figshare (see Data Availability Statement). Since composite phylogenies do not have true branch lengths, we used Grafen's method (Grafen 1989) to generate branch lengths using the 'ape' package (Paradis and Schliep 2019). In each model we used the phylogenetic signal (i.e., Pagel's λ) as estimated by the maximum-likelihood method for that model. In each PGLS model, the dependent variable was one of the 16 WorldClim variables, and the fixed effects were the type of GSD (XX/XY or ZZ/ZW), the type of TRN (MF, FMF, or FMF), and their interaction. Note that the interaction was included so that the model can estimate the effect of GSD type within each TRN type; our predictions refer to these effects and not the interaction. Because the FMF reaction norm is not known to occur in any amphibian families, we analysed amphibians and reptiles separately. Due to differences in sample size, we allowed for variances to differ between groups (among the four combinations of GSD and TRN in amphibians, and among the three TRN types in reptiles; we could not estimate variances for each combination of GSD and TRN in reptiles because one of these combinations had no variance, i.e., $N=1$). To test our predictions, we used pre-planned comparisons and reported the effect sizes with 95% confidence intervals (corresponding to a significance level of 5%) as recommended for evolutionary-ecological studies with multiple comparisons (Nakagawa 2004; Ruxton and Beauchamp 2008). From each model, we estimated the difference (linear contrast) between XX/XY and ZZ/ZW systems within each type of TRN using the 'emmeans' function of the 'emmeans' package (Lenth 2023). We present the detailed results of all models in Table S1.

We analysed the WorldClim variables to maximise sample size, as these data were available for all species in our dataset ($N=208$) while we had SexClim data for a smaller subset ($N=120$). To assess how well each WorldClim variable may reflect the variation across species in the temperatures of the sex-determining period, we tested the pairwise Pearson correlation between each of the 16 WorldClim variables and each of those SexClim variables that referred to the same aspect of climatic distribution (e.g., mean, median, etc.) separately for amphibians and reptiles (Table S2).

2.5 | Thermal Thresholds

We collected data on thermal thresholds from the HerpSexDet database (Nemesházi and Bókony 2023) defined as the temperature at which the offspring sex ratio becomes significantly skewed towards one sex. We took these data from those taxonomic families that met the following criteria: (1) TRN data were available and TRN patterns reported in the family were non-contradictory, (2) thermal thresholds were available from more than one species (or hybrids), with at least one data point for lower and upper threshold each and (3) we had climatic-range data for both XX/XY and ZW/ZZ species. Because of small sample sizes, we did not analyse these data statistically; instead, we visualised them in graphs to qualitatively compare the climatic distributions of XX/XY and ZW/ZZ species to the reported thermal thresholds in each taxonomic family.

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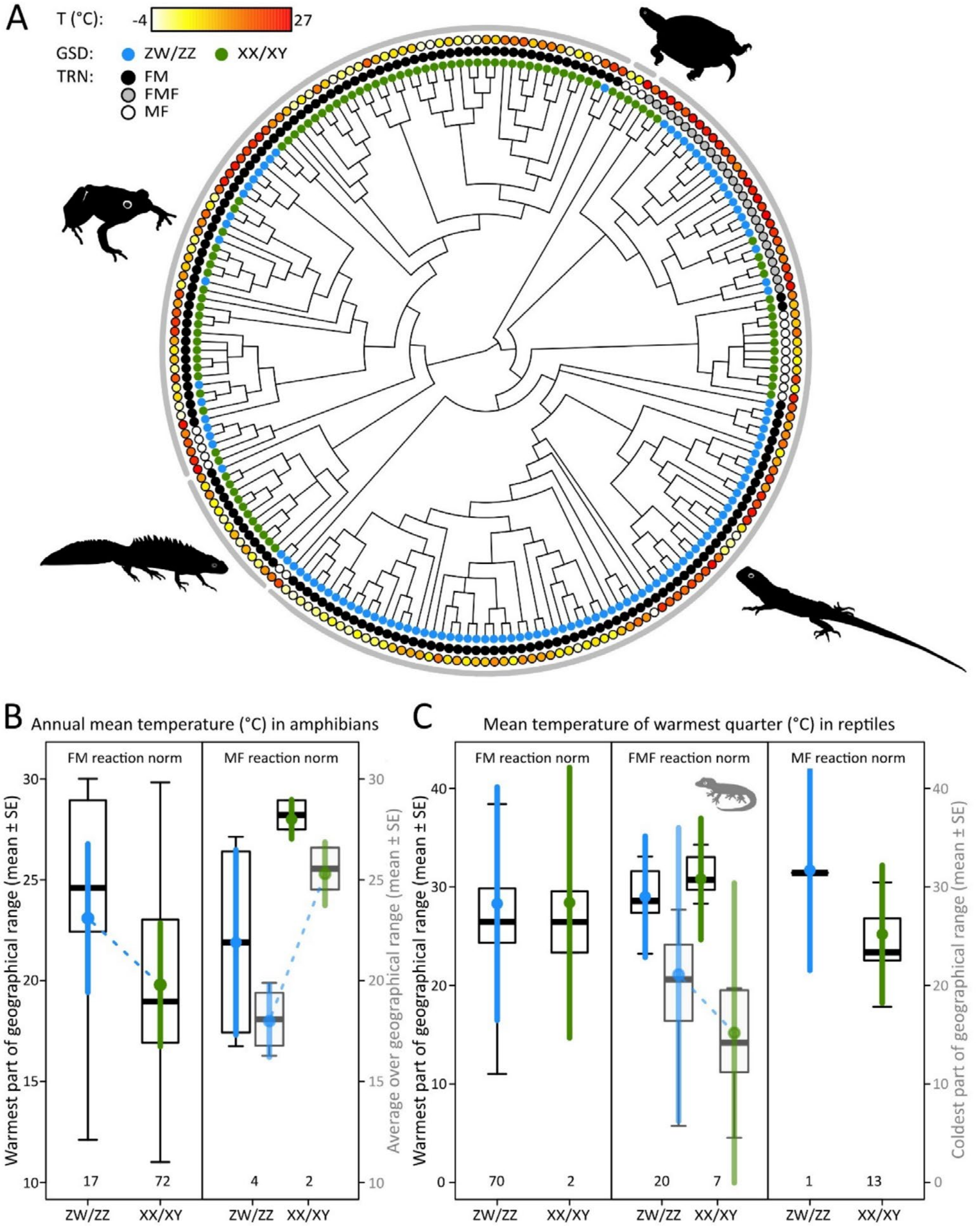

FIGURE 4 | Phylogenetic relationships between climatic distribution, genotypic sex determination (GSD), and thermal reaction norm (TRN) in herpetofauna. Across the phylogeny (A), climate for each species is illustrated as the average annual mean temperature (T) over the geographical distribution range. The variable that correlates best with the temperature of the sex-determination period (black boxes with white fill) is the annual mean temperature in the warmest part of the distribution range (abbreviated as 'BIO1 max' in Figure 5) for amphibians (B), and the mean temperature of the warmest quarter of the year in the warmest part (BIO10 max) of the distribution range for reptiles (C). Differences that support the theory of asymmetrical sex reversal are highlighted by dotted lines ($p < 0.048$). Additionally, dark grey boxes with light grey fill illustrate the annual mean temperature averaged over the distribution range (BIO1 mean) for amphibians (B), and the mean temperature of the warmest quarter of the year in the coldest part of the distribution range (BIO10 min) for reptiles (C). These additional variables are shown because they correlate best with the temperature of the sex-determination period among those variables that showed a significant difference between GSD types in MF amphibians and in FMF reptiles (see Figure 5). Coincidentally, these variables are the third best-correlating proxy for the sex-determination period in each class (see Table S2). In each box plot (B, C), the thick middle line, box, and whiskers represent the median, interquartile range, and data range, respectively; the coloured dots with error bars depict the mean and ±SE estimated from phylogenetic generalised least-squares models, and the number below each plot is the number of species for which sex-determination data are available. Figure panels were arranged and artwork was added in INKSCAPE 1.2.

3 | Results

Data from HerpSexDet (Nemesházi and Bókony 2023) enabled the assignment of TRN patterns to 208 species with known GSD type across the phylogeny of herpetofauna (Figure 4A) distributed across all continents except Antarctica (Figure 3). The number of species for which sex-determination data were available differed between TRN-GSD combinations (Figure 4b,c).

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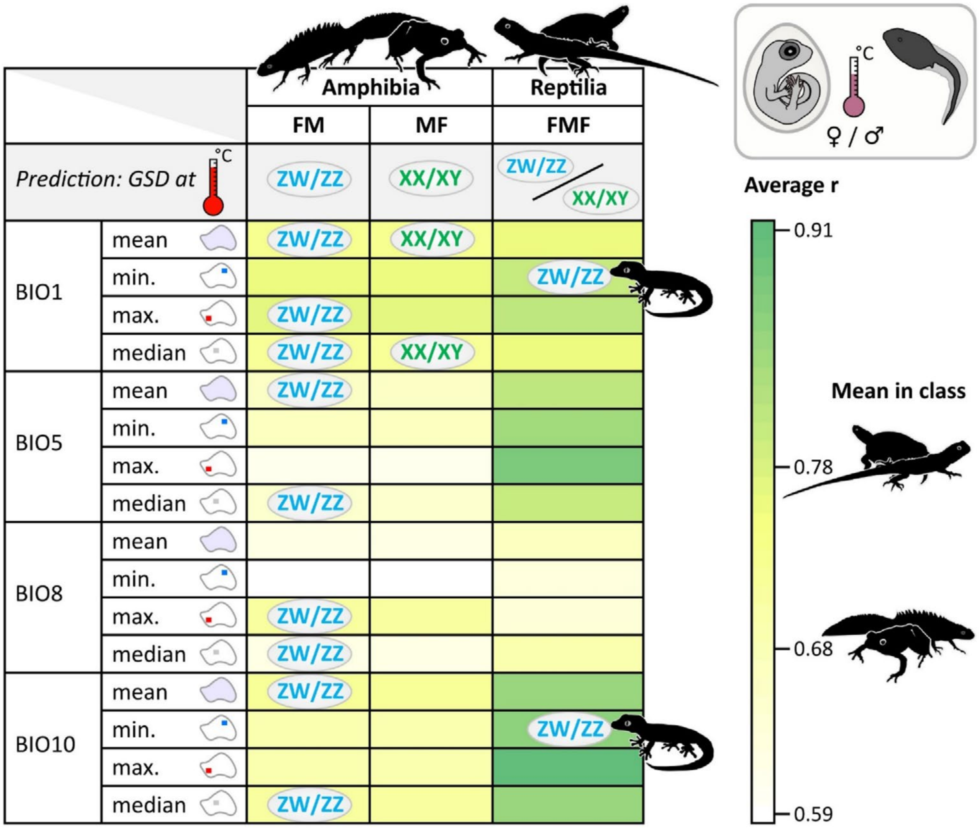

		Amphibia FM	Amphibia MF	Reptilia FMF
Prediction: GSD at °C		ZW/ZZ	XX/XY	ZW/ZZ / XX/XY
BIO1	mean	ZW/ZZ	XX/XY	
	min.			ZW/ZZ
	max.	ZW/ZZ		
	median	ZW/ZZ	XX/XY	
BIO5	mean	ZW/ZZ		
	min.			
	max.			
	median	ZW/ZZ		
BIO8	mean			
	min.			
	max.	ZW/ZZ		
	median	ZW/ZZ		
BIO10	mean	ZW/ZZ		
	min.			ZW/ZZ
	max.			
	median	ZW/ZZ		

FIGURE 5 | Overview of the results of the 16 models and their estimated relevance for environmental temperatures around the sex-determining period. Grey ovals represent significant differences between the sex-chromosome systems (detailed as 'Pairwise comparisons' in Table S1 for each TRN pattern in each PGLS model), and the text in the ovals refers to the system that occurs at warmer temperatures. Polygons for each WorldClim variable illustrate how we calculated the mean, minimum (i.e., coldest area), maximum (i.e., warmest area), and median values over the native distribution range for annual mean temperature (BIO1), maximum temperature of the warmest month (BIO5), mean temperature of the wettest quarter (BIO8), and mean temperature of the warmest quarter (BIO10). Cell background colours indicate the correlation coefficients (Pearson's *r*) averaged across corresponding SexClim variables (i.e., temperature variables calculated for the approximate sex-determining period for a subset of species) for each WorldClim variable in each taxonomic class.

Asymmetrical sex reversal predicts that, under FM TRN pattern, ZW/ZZ systems should be found in warmer environments than XX/XY systems. In amphibians, nine out of 16 PGLS models supported this prediction, showing that species with the FM reaction norm occupied significantly warmer areas if they had the ZZ/ZW system compared to the XX/XY system (Figure 5; see details in Table S1, particularly the 'Pairwise comparisons' from each model). Among species with the MF reaction norm, asymmetrical sex reversal predicts that XX/XY systems should be found in warmer environments than ZW/ZZ systems; two models supported this prediction for amphibians (Figure 5; Table S1). Overall, there was a strong correlation between WorldClim and SexClim variables, with coefficients ranging 0.567–0.806 (Table S2). The climatic variable best correlating with the temperature of the sex-determining period across amphibians was the annual mean temperature in the warmest part of the distribution range (Table S2); this variable supported the predicted climatic differences between GSD types for FM species (Figure 4B). The estimated temperatures of the sex-determining period had relatively strong correlations (i.e., stronger than the average across the 16 variables in amphibians) with most (six out of nine) climatic variables that showed the difference predicted for the FM pattern, and with both variables that supported our predictions for the MF pattern (Figure 5; Table S2).

In reptiles, there were no differences between the two GSD systems among species with either FM or MF reaction norms (Figure 4C, Table S1). Unexpectedly, in two out of 16 PGLS models, the coldest area of the distribution range was significantly warmer among species with the FMF reaction norm if they had the ZZ/ZW system compared to the XX/XY system (Figure 4C, Figure 5; see 'Pairwise comparisons' from each model in Table S1). Notably, all species with FMF reaction norm in our dataset were geckos from infraorder Gekkota. Correlation coefficients between WorldClim and SexClim variables ranged 0.595–0.950 (Table S2). The climatic variable that best correlated with the temperature of the sex-determining period across reptiles was the mean temperature of the warmest quarter of the year in the warmest part of the distribution range (Table S2). However, the latter variable did not support any of the predicted effects of GSD and TRN (Table S1, Figure 5; Figure 4C). The two climatic variables that showed a difference between GSD types within the FMF pattern had relatively strong correlations (i.e., stronger than the average across the 16 variables in reptiles) with the estimated temperatures of the sex-determining period (Figure 5; Table S2).

Altogether 3 amphibian and 1 reptile families met our criteria for qualitative comparison of species-level climatic data to

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thermal thresholds of significant sex-ratio bias reported in their respective families. These data show that both FM and MF amphibians and FMF geckos tend to occupy those thermal habitats that are expected not to cause sex reversal in their respective sex-determination systems (Figure S1).

4 | Discussion

Our results demonstrate that the climatic distribution of at least some ectothermic amniote species can be explained by the interplay between genotypic sex determination and clade-specific temperature reaction norm of sexual development, and for amphibians the observed differences conform to the predictions derived from the theory of asymmetrical sex reversal. These findings have several implications. First, they indicate that temperature-induced sex reversal may be an important driver of phylogeography and evolution of sex-determination systems across a wide range of species. Second, as contemporary climate change proceeds, the importance of temperature-induced sex reversal may increase, and the types of both GSD and TRN may determine which species would be most affected by its consequences, including climatic range shifts and even population extinctions. Finally, the different results we found in amphibians versus reptiles may either reflect true biological differences or may be simply due to variation in available sample size and thus statistical power. In either case, our study highlights the pressing need for collecting empirical data on GSD and especially TRN in many more species from a broad taxonomical spectrum, for a better understanding of both past and future evolutionary processes and conservation priorities.

In accordance with our predictions, we found that amphibian species with ZW/ZZ systems occupied significantly warmer areas than those with XX/XY systems in clades with FM TRN pattern, while the opposite pattern was found in clades with MF pattern. These differences were found with several climatic variables, which were not a random subset of the variables we investigated: mostly they showed higher than average correlation with temperature variables measured within the period of larval development in amphibians. These differences not only follow the predictions of asymmetrical sex reversal, but to our knowledge, no other current theory explains them. Further supporting evidence comes from taking a closer look at the climatic ranges occupied by species in comparison with thresholds of significant thermal sex-ratio bias in their taxonomic families (Figure S1). Among those taxonomic families where we could make this comparison, two amphibian families belong to clades with FM TRN pattern (Ranidae and Dicroglossidae). Qualitative comparison of our results on relevant climatic variables to the average temperature thresholds in these families indicates two interesting trends. First, the occupied temperature ranges usually fell between the lower and upper thresholds of sex-reversing temperature ranges during 1970–2000 (the period of climatic data downloaded from the WorldClim 2 database). Second, compared to ZW/ZZ, XX/XY species occurred more often at male-to-female sex-reversing temperatures, where sex reversal is not expected to happen due to resistance in XY individuals (Figure S1). However, at the warmest parts of their geographic ranges, species in these two anuran families experienced average annual temperatures much closer to female-to-male sex-reversing temperatures already during 1970–2000 (Figure S1), and temperature increase of the past decades might explain why sex-reversed XX males can be found in large proportions in some populations of multiple species in Ranidae (Alho et al. 2010; Lambert et al. 2019; Nemesházi et al. 2020; Xu et al. 2021). The MF pattern was found in only a few amphibian species, all but one of which belong to a single anuran family (Pipidae). In this family, both XX/XY and ZW/ZZ species occupied habitats with temperatures remarkably close to those respective thermal thresholds that are expected not to cause sex reversal in them (Figure S1). This corroborates our main findings for amphibians with the MF pattern, despite the very small sample sizes available for this group (i.e., ≤ 4 species for each GSD system).

In contrast to our results in amphibians, we found none of the predicted differences in climatic ranges between GSD systems among reptiles with either FM or MF pattern. The simplest explanation for these contrasting results may lie in the heterogeneity of sample sizes available from the literature. The comparison most persistently supported by our analyses was the one for which we had the highest power (i.e., $N > 15$ species for each GSD system): within FM amphibians. For reptiles, all three comparisons had limited power because the number of species was very low (≤ 7) for one of the two GSD systems within each comparison. One reason for this scarcity of data is that different clades tend to receive attention from different points of view: some are studied for temperature effects on sexual development, while others are being researched for the presence of sex chromosomes. However, an alternative explanation is that reptiles may truly be less conforming to asymmetrical sex reversal compared to amphibians. Potential reasons for this include maternal thermoregulation (e.g., basking; Dubiner et al. 2024) and nest-site choice (Doody et al. 2006) in viviparous and oviparous reptiles, respectively, which may allow for higher control over the thermal environment of their offspring compared to amphibians where free-moving aquatic larvae are most typical (Liedtke et al. 2022). However, aquatic larvae may also express stage-dependent temperature preferences within the limitations of their microhabitat (Catenazzi and Kupferberg 2013; Floyd 1984). To confront these alternatives and to test the generality of the asymmetrical sex-reversal theory, we urge empirical work especially on species in clades where GSD occurs and the effects of temperature on sexual development have not yet been tested. Because temperature-induced sex reversal can occur not only in herpetofauna but also in other taxa like fish (Geffroy and Wedekind 2020; Ospina-Álvarez and Piferrer 2008; Valdivia et al. 2014), studies of marine and freshwater temperatures across various water depths could further clarify the role of temperature-induced sex reversal in the phylogeography and evolution of GSD species.

Unexpectedly, we found in reptiles with FMF TRN pattern that, during the warmest quarter of the year (the period with highest overall correlation with environmental temperatures during embryonic development in reptiles; see Figure 5), the coldest parts of the geographical distribution ranges of ZW/ZZ species were significantly warmer compared to those of XX/XY species. This difference resembles the pattern expected under FM reaction norm, and thus it might suggest that environmental temperatures of the studied species in clades with FMF are not yet so high that would induce male-to-female sex reversal. Instead,

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these species may currently experience temperatures around the lower threshold: i.e., female-producing temperatures below and male-producing temperatures above this threshold. In our dataset, the FMF TRN pattern was only assigned to the gecko families Eublepharidae and Gekkonidae, due to the lack of GSD data in other FMF species and to the ambiguity of TRN patterns in other families where FMF occurs. We had data on the timing of early embryonic development for some of these species, and the mean temperature across their natural distribution range during this period was indeed closer to the lower temperature threshold (data for Gekkonidae are shown in Figure S1), conforming to the above suggestion. It is therefore possible that temperature-induced male-to-female sex reversal might predominantly occur at the coldest parts of the geographical distribution ranges in the studied gecko species, contributing to the temperature difference between species with XX/XY and ZW/ZZ systems found here. However, empirical data are currently missing to verify this explanation. Interestingly, the temperatures in the warmest parts of these geckos' distribution ranges coincide with the male-producing range of their FMF reaction norm (Figure S1), where temperature differences between XX/XY and ZW/ZZ species show a reversed trend compared to the coldest parts (Figure 4C), which also conforms to the predictions of asymmetrical sex reversal.

Our results highlight the importance of collecting data on the occurrence of sex chromosomes in parallel with data on the effects of temperature on sexual development in ectothermic vertebrate species. Empirical studies so far, especially in reptiles, have predominantly focused on either GSD or TSD, and once a species was categorised as either GSD or TSD, researchers rarely attempted to evaluate if genotypic sex and environmental temperatures could both influence sexual development; although such rare attempts revealed cryptic patterns (Cornejo-Páramo, Lira-Noriega, et al. 2020; Dissanayake, Holleley, Deakin, et al. 2021; Geffroy et al. 2021; Holleley et al. 2015; Ujszegi et al. 2022). Moreover, various studies assessing TRN applied only a limited number of incubation temperatures and made conclusions on limited sample sizes (see 'TSD_note' in HerpSexDet (Nemesházi and Bókony 2023) and see also Ospina-Álvarez and Piferrer 2008). In such studies it is difficult to infer if sex-ratio bias was caused by sex reversal or sex-biased mortality, which is a common methodological constraint (Nemesházi and Bókony 2022). We suspect that these limitations are largely responsible for the significant lack of fundamental empirical information regarding the potential temperature-sensitivity of sexual development under GSD. Besides conducting laboratory experiments, it is important to uncover sex-reversal frequencies in wild populations as well, by comparing the genotypic sex with the phenotypic sex in each captured individual (Dissanayake, Holleley, Deakin, et al. 2021; Holleley et al. 2015; Nemesházi et al. 2020; see further reports in Nemesházi and Bókony 2023). Such studies should account for the possibility that certain chemical pollutants may also induce sex reversal (see examples in Nemesházi and Bókony 2022). Collecting such empirical data is crucial for understanding how and to what extent environmental conditions influence the evolution of sex determination and its consequences for demography and population persistence.

While previous empirical studies suggested that GSD and TSD systems in reptiles are associated with different climatic conditions (Cornejo-Páramo, Lira-Noriega, et al. 2020; Valenzuela and Adams 2011), here we demonstrate for the first time in amphibians and some reptiles that within GSD, even XX/XY and ZW/ZZ systems can be associated with different environmental temperatures. Our results on the climatic distribution of XX/XY and ZW/ZZ systems suggest that, over the evolutionary past, thermal sex reversal might have driven a 'co-evolution' of the species' geographical ranges and sex determination, i.e., the combination of sex chromosomes and thermal reaction-norm thresholds, via the pathways illustrated in Figure 2. This implies that ongoing and future directional temperature changes (either via climate change, geographical range shifts or both) may influence transitions between different sex-determination systems in a non-random way, favouring one sex-chromosome system over the other, depending on the shape of the TRN featured by the given taxon. Evaluating if species are able to adapt fast enough to currently accelerating climatic changes is one of the most pressing biodiversity-conservation problems. We propose that taking asymmetrical sex reversal into account can help to pinpoint the taxa most vulnerable to rising environmental temperatures.

Author Contributions

E.N. conceived the concept of the study, carried out data collection, calculated the climatic variables and wrote the first draft of the manuscript. V.B. fine-tuned the study goals, carried out the statistical analyses and revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data and code that support the findings of this study are available in the Supporting Information of this article and in Figshare: https://doi.org/10.6084/m9.figshare.25624983.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1.** Three temperature variables and thresholds of thermal sex-ratio bias. **Table S1.** Results of phylogenetic least squares (PGLS) models for each climatic variable. **Table S2.** Pairwise Pearson correlation coefficients. **Table S3.** Justification for the categorization of TRN patterns.

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