

RESEARCH PAPER

Are evolutionary transitions in sexual size dimorphism related to sex determination in reptiles?

Gergely Katona¹  | Balázs Vági¹  | Zsolt Végvári^{2,3}  | András Liker^{4,5}  |
Robert P. Freckleton⁶  | Veronika Bókony⁷  | Tamás Székely^{1,8,9,10} 

¹Department of Evolutionary Zoology and Human Biology, University of Debrecen, Debrecen, Hungary

²Centre for Ecological Research, Eötvös Loránd Research Network, Danube Research Institute, Budapest, Hungary

³Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany

⁴MTA-PE Evolutionary Ecology Research Group, University of Pannonia, Veszprém, Hungary

⁵Behavioural Ecology Research Group, Center for Natural Sciences, University of Pannonia, Veszprém, Hungary

⁶Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK

⁷Lendület Evolutionary Ecology Research Group, Plant Protection Institute, Centre for Agricultural Research, Eötvös Loránd Research Network, Budapest, Hungary

⁸Milner Centre for Evolution, University of Bath, Bath, UK

⁹State Key Laboratory of Biocontrol, Department of Ecology, School of Life Sciences, Sun Yat-sen University, Guangzhou, China

¹⁰Ministry of Education Key Laboratory for Biodiversity and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing, China

Correspondence

Gergely Katona, Department of Evolutionary Zoology and Human Biology, University of Debrecen, H-4032 Debrecen, Hungary.
Email: gergelykatona88@gmail.com

Funding information

Development and Innovation Office of Hungary, Grant/Award Number: K 116310, K-115402 and PD 132819; ÉLVONAL, Grant/Award Number: KKP-126949

Abstract

Sex determination systems are highly variable in vertebrates, although neither the causes nor the implications of this diversity are fully understood. Theory suggests that sex determination is expected to relate to sexual size dimorphism, because environmental sex determination promotes sex-specific developmental bias in embryonic growth rates. Furthermore, selection for larger size in one sex or the other has been proposed to drive the evolution of different genetic sex determination systems. Here, we investigate whether sex determination systems relate to adult sexual size dimorphism, using 250 species of reptiles (Squamata, Testudines and Crocodylia) representing 26 families. Using phylogenetically informed analyses, we find that sexual size dimorphism is associated with sex determination: species with TSDIa sex determination (i.e. in which the proportion of female offspring increases with incubation temperature) have more female-biased size dimorphism than species with TSDII (i.e. species in which males are produced at mid temperatures). We also found a trend that species with TSD ancestors had more male-biased size dimorphism in XY sex chromosome systems than in ZW sex chromosome systems. Taken together, our results support the prediction that sexual size dimorphism is linked to sex-dependent developmental variations caused by environmental factors and also by sex chromosomes. Since the extent of size dimorphism is related to various behavioural, ecological and life-history differences between sexes, our results imply profound impacts of sex determination systems for vertebrate diversity.

KEYWORDS

development, environmental sex determination, genetic sex determination, phylogenetic comparative methods, sexual size dimorphism

Gergely Katona and Balázs Vági have equal contribution to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Journal of Evolutionary Biology* published by John Wiley & Sons Ltd on behalf of European Society for Evolutionary Biology.

1 | INTRODUCTION

Vertebrates exhibit various sex determination systems (Bachtrog et al. 2014; Beukeboom & Perrin, 2014). The processes that created this diversity constitute some of the major unresolved issues in evolutionary biology. In genetic sex determination systems (GSD), offspring sex is determined by genetic factors at conception (Ellegren, 2000; Scharlt et al. 2004). The most widespread form is when one of the sexes carries heterogametic sex chromosomes. For example, in XY systems, the males represent the heterogametic sex, whereas in ZW systems, these are the females. In environmental sex determination (ESD) systems, however, the sex of individuals is determined by ambient environmental factors, often the ambient temperature (temperature-dependent sex determination, TSD) during embryonic development (Valenzuela & Lance, 2004). TSD systems are conventionally classified into three sub-types: TSDIa, where females develop at higher temperatures and males develop at lower temperatures; TSDIb, where the pattern is reversed; and TSDII, where females develop at both lower and higher temperatures, whilst males develop at intermediate temperatures (reviewed by Valenzuela & Lance, 2004). Although mixed sex determination systems exist (where sex depends on both genetic and environmental factors), most tetrapods (i.e. amphibians, reptiles, birds and mammals) appear to exhibit either GSD or ESD (Bachtrog et al. 2014; Beukeboom & Perrin, 2014).

Variation in sex determination systems has been linked to a multitude of extrinsic and intrinsic factors, for example ecological patterns such as variation in ambient temperature (Cornejo-Páramo et al. 2020; Pen et al. 2010), life-history parameters such as longevity (Janzen & Paukstis, 1991a,b; Sabath et al. 2016), reproductive mode (Organ et al., 2009) and demographic composition of the populations expressed as adult sex ratio (Pipoly et al. 2015). A link between sex determination and sexual size dimorphism is also suggested, either by direct physiological causation or indirectly, acting through the above-mentioned mediators (Adkins-Regan & Reeve, 2014; Ewert et al. 1994; Ewert & Nelson, 1991; Janzen & Paukstis, 1991a,b; Kraak, 1994; Schwanz et al. 2016; Viets et al. 1994).

Temperature-dependent sex determination is thought to be adaptive when developmental temperatures act differentially on the fitness of the two sexes (Charnov & Bull, 1977; Ewert & Nelson, 1991; Schwanz et al. 2016). This hypothesis was experimentally confirmed in a short-lived agamid lizard *Amphibolurus muricatus*, where the lifetime reproductive success in each sex was maximized by the developmental temperature producing that sex (Warner & Shine, 2008). In this species, TSD causes a seasonal shift in the adult sex ratios as early-hatching females have a higher chance to successfully reproduce within the same season than early-hatching males that are usually unsuccessful in intrasexual competition in their first year. The sex-specific influence of developmental temperatures on individual fitness is likely mediated by their effects on body size. Whilst in some species males may get more benefits from larger size in competition (Rohner et al. 2016; Székely et al. 2004; Warner & Shine, 2008), in other species females may benefit more from larger

adult body size due to fecundity selection (Fairbairn et al. 2007; Pincheira-Donoso et al. 2017; Serrano-Meneses & Székely, 2006).

Theory postulates that evolution should favour the type of TSD that ensures that the sex benefiting most from larger size develops at temperatures conferring larger size (Schwanz et al. 2016). This hypothesis predicts larger females in species with TSDIa and larger males in species with TSDII. Although previous studies have investigated this proposition by comparing multiple vertebrate species (Ewert et al. 1994; Ewert & Nelson, 1991; Janzen & Paukstis, 1991a,b), they produced contradicting results and often did not control for phylogenetic relatedness amongst the species in statistical analyses. Recently, new data on sex determination systems and on sexual size dimorphism have become available, together with better phylogenies. These allow for more accurate phylogenetically controlled tests of this fundamental prediction of TSD theory than those conducted in earlier studies.

The evolution of GSD has been also hypothesized to be driven by selection for sexual size dimorphism. Kraak and de Looze's (1994) model predicts that if ancient Y and W chromosomes enhance growth rate and this trend is favoured by selection, then XY systems with larger males and ZW systems with larger females should evolve from ESD. Adkins-Regan and Reeve's (2014) model is consistent with this logic and predicts that a masculinizing allele is favoured by selection and leads to the development of XY systems when larger males have an evolutionary advantage in an ancestral ESD system with female-biased dimorphism, whereas a feminizing allele leading to ZW system is favoured when larger females are selected for and the ancestral state is ESD with male-biased dimorphism. Thus, Adkins-Regan and Reeve (2014) predict that male-biased dimorphism should emerge for XY species and female-biased dimorphism for ZW species evolving from ancestral ESD systems. By analysing 26 reconstructed evolutionary transitions from ESD to XY or ZW systems, Adkins-Regan and Reeve (2014) found support for their model in ectothermic vertebrates, although their relevant analysis was mostly restricted to fishes, and included only two species-pairs of reptiles.

Nonavian reptiles (lizards, snakes, turtles and crocodiles, approx. 10,000 species) have diverse sex determination systems (Pokorná et al. 2016). Although nonavian reptiles do not constitute a monophyletic group, all birds have ZW sex determination and their physiology (endothermy and metabolic rate) is characteristically different from other Sauropsida, posing different physiological constraints on the evolution of sex determination and sexual size dimorphism. In contrast, in nonavian reptiles (henceforth: reptiles), the different types of sex determination are not restricted to a single clade because several evolutionary transitions have occurred (Pokorná & Kratochvíl, 2008; Gamble et al. 2015; Sabath et al. 2016). This diversity coupled with rich data on their natural history, and phylogenetic relationships make reptiles an excellent model group for studies of sexual size dimorphism. Despite this potential, previous studies on the associations between sex determination systems and sexual size dimorphism were limited either in their taxonomic scope and/or sample size, and were constrained by the availability of robust phylogenetic hypotheses (Adkins-Regan & Reeve, 2014; Ewert

et al. 1994; Ewert & Nelson, 1991; Janzen & Paukstis, 1991a,b; Kraak & de Looze, 1994).

Here, we evaluate whether sex determination system relates to sexual size dimorphism in adult body size amongst reptiles using data from 250 extant species from 26 families of Squamata, Testudines and Crocodylia. First, we investigate whether amongst-species variance in sexual size dimorphism differ between ESD and GSD types. Because TSD reptiles exhibit more interspecific variation in sex differences in maturation age than GSD reptiles (Bókonyi et al. 2019), we expect larger variation in sexual size dimorphism amongst the former than the latter. Second, we investigate whether sexual size dimorphism differs between sex determination types. Specifically, we expect that TSDIa species should exhibit more female-biased sexual size dimorphism than TSDII species. Furthermore, based on Adkins-Regan and Reeve (2014), we expect more male-biased sexual size dimorphism in XY species than in ZW species if their ancestral state was ESD.

2 | MATERIAL AND METHODS

2.1 | Data collection

We collected sex-specific mean adult body length data from peer-reviewed journals, online databases and books, as well as by retracing original sources cited in reviews (see Supplementary Material S1). In lizards, snakes and crocodylians, we collected snout to vent length (SVL – measured from the tip of the nose to the cloaca) data. In turtles, we used either carapace length or plastron length as widely accepted metrics of body length, depending on data availability in the literature. Body size dimorphism was calculated from adult body length data as \log_{10} (male body length/female body length). Mean body size was also calculated as the mean of male and female adult body length. For some species ($N = 64$), we found body length data for more than one population; in these cases, we calculated the species' sexual size dimorphism values for each population and then averaged the sexual size dimorphism values for the species.

Sex determination data were collated using the online database Tree of Sex (The Tree of Sex Consortium, 2014), which we supplemented with data from more recent literature (Supplementary Material S1). Due to their limited number, we excluded species with TSDIb sex determination ($N = 6$ reptile species) from our analyses. We also excluded species with mixed sex determination (i.e. where both GSD and TSD have been reported). Overall, 250 species were used for which both sex determination type and data on sexual size dimorphism were available (see Supplementary Material S1).

2.2 | Statistical analysis

We carried out a repeatability analysis using the R package “rptR” (Stoffel et al. 2017) to ensure that our data are representative of the species' mean body size dimorphism (i.e. repeatable within species

between the populations). Repeatability (R) is given as the intra-class correlation coefficient with 95% confidence interval (CI). Sexual size dimorphism had a high intra-class correlation between populations of a given species ($R = .824$, $CI = 0.726–0.888$, $p = .014$, $N = 64$ species, $N = 128$ populations).

To account for phylogenetic nonindependence amongst species, we performed phylogenetically controlled analyses. We used a reptile phylogenetic tree retrieved from the NCBI-based database of phylogenetic relationships utilized by TimeTree (Kumar et al. 2017) to provide the same phylogeny construction approach for all three reptile clades in our analyses. We also added species missing from this tree ($N = 44$ species) to our phylogeny, pairing them to their closest known sister species within their genus, based on a literature search of phylogenetic studies of the species group (Pyrón et al. 2013).

We analysed our data applying phylogenetic generalized least-squares (PGLS) models (Freckleton et al. 2002) in the R statistical programming environment (R-Core-Team, 2013). In these models, we used the type of sex determination as a factor with two categories (the categories varied across analyses as detailed below; see Table 1). We compared the amongst-species variance of sexual size dimorphism between GSD and ESD species using the “glS” function in “nlme” package (Pinheiro et al. 2020), which accommodates different variances between groups as well as phylogenetic control. We built two models, in one of which we allowed within-group (between-species) variance in sexual size dimorphism to differ between GSD and ESD, and another model in which variance was assumed to be homogeneous. To test whether variance in sexual size dimorphism differs between GSD and ESD species, we compared model fit between the two models with a likelihood ratio test (Bolker et al. 2009). Additionally, with a similar test design we compared variance in sexual size dimorphism among major clades of reptiles (Crocodylia, Testudines and Squamata), and also between ESD and GSD species within each of the two clades that include both ESD and GSD species, that is turtles and squamates.

To test whether sexual size dimorphism differs between pairs of sex determination types, we carried out PGLS analyses applying the package “caper” (Orme et al. 2013). In our first model, we compared sexual size dimorphism between TSDIa and TSDII species. To exclude the potential distorting effect of extreme values in sexual size dimorphism, we generated 30 replicates of this analysis by randomly sampling 90% of the species. Also, we repeated the comparison within turtles, the only clade that includes both TSDIa and TSDII species. In our second model, genetic sex determination systems ZW and XY were compared. In the third model, we compared ZW and XY species again, but restricted the analysis to GSD species which have ESD ancestry (see below). Since sexual size dimorphism often relates allometrically to body size (Rensch's rule, Székely et al. 2004), in an additional set of analyses we controlled for the potential effect of body size by including log-transformed mean body size in the above three PGLS models.

We identified those species where the current state of GSD originated from ESD according to an ancestral state reconstruction of

TABLE 1 Sexual size dimorphism (SSD; response variable) in relation to sex determination systems (SD) in reptiles (phylogenetic least-squares models)

Model	Explanatory variable(s)	λ	T	df	adjusted R ²	p
Model 1 SSD ~ SD	TSD type (TSDIa, TSDII)	0.417	2.60	85	0.063	.01
Model 2 SSD ~ SD + BS	TSD type (TSDIa, TSDII)	0.478	2.41	85	0.044	.018
	Body size		0.19	85		.85
Model 3 SSD ~ SD	GSD type (XY, ZW)	0.93	0.463	157	0.004	.643
Model 4 SSD ~ SD + BS	GSD type (XY, ZW)	0.93	0.184	157	0.008	.854
	Body size		1.831	157		.068
Model 5 SSD ~ SD	GSD type (XY, ZW) with ESD ancestry	0.00	1.02	17	0.083	.32
Model 6 SSD ~ SD + BS	GSD type (XY, ZW) with ESD ancestry	0.00	1.8	17	0.14	.09
	Body size		1.42	17		.173
Model 7 SSD ~ SD	GSD type (XY, ZW) with ESD ancestry	0.00	1.17	14	0.083	.32
Model 8 SSD ~ SD + BS	GSD type (XY, ZW) with ESD ancestry	0.00	3.73	14	0.542	.002
	Body size		2.66	14		.02

Note: ESD and GSD refer to environmental and genetic sex determination, respectively, BS refers to average body size of males and females.; λ is the maximum likelihood estimate of phylogenetic signal. The bold values represents significant values. Number of TSDIa and TSDII species, respectively: 52 and 36 in models 1–2. Numbers of XY and ZW species, respectively: 56 and 101 in models 3–4; 5 and 14 in models 5–6; 5 and 11 in models 7–8.

species with known sex determination systems, which we carried out using stochastic character mapping, as implemented by the “ace” function available in the R package “phytools” (Revell, 2012), using maximum likelihood estimation for 4 discrete characters of sex determination (TSDIa, TSDII, XY and ZW) (Supplementary material S2, Figure S1). Species were identified as having TSD ancestral state when TSDIa or TSDII had more than 0.8 likelihood in one of their ancestral nodes, and only one transition was detected between the ancestral ESD and the current GSD state.

3 | RESULTS

Sex determination is variable in both turtles (from Trionychidae to Podocnemidae) and lizard clades including Gekkota (from Phyllodactylidae to Carphodactylidae), Lacertoidea (from Lacertidae to Gymnophthalmidae) and Iguania (from Dactyloidae to Chamaeleonidae; Figure 1). There is no variation in sex determination in crocodylians (Crocodylidae, Gavialidae and Alligatoridae), where all species have TSDII in our dataset, and little variation is present in snakes (represented by Boidae, Viperidae, Elapidae and Colubridae, Figure 1) since vast majority of species have ZW systems. The extent and direction of sexual size dimorphism are highly variable within most clades of turtles, snakes and lizards, with female-biased dimorphism being more frequent in turtles and male-biased dimorphism being more frequent amongst lizards. In contrast, crocodylians either have male-biased dimorphism or exhibit monomorphism (Figure 1).

The variance in sexual size dimorphism is significantly higher amongst ESD species than amongst GSD species (likelihood ratio test $\Delta AIC = 37.1$, $p < .01$). We found significant difference in the variance of sexual size dimorphism amongst the major reptile clades:

both squamates and turtles have larger variance than crocodylians (likelihood ratio test; $\Delta AIC = 103$, $p < .01$, Supplementary material S2, Figure S3). In turtles, amongst-species variance differs significantly between ESD and GSD, with larger variance in ESD species (likelihood ratio test; $\Delta AIC = 2$, $p = .04$, Supplementary material S2, Figure S4). In contrast, the variance between ESD and GSD in squamates is not significantly different (likelihood ratio test; $\Delta AIC = 5$, $p = .6$, Supplementary material S2, Figure S5).

In ESD species, sexual size dimorphism is significantly different between the types of sex determination, since in TSDIa species females are on average larger than the males, whereas TSDII species usually have larger males than females (Figures 2 & 3a, Table 1). Body size is unrelated to sexual size dimorphism, and including body size into the model does not change qualitatively the association between sexual size dimorphism and the type of ESD (Table 1: models 1 & 2). After random sampling of 90% of ESD species, in 29 out of 30 analyses the difference between ESD types remains significant ($p = .01 \pm 0.029$; Table S1). In turtles ($N = 59$), the only group where both TSDIa and TSDII are present, the relationship between ESD type and sexual size dimorphism is not significant ($F_{56} = 0.75$, $p = .38$); however, the direction of the difference is the same as the general pattern in all reptiles (Supplementary material S2, Figure S6).

In GSD species, sexual size dimorphism is not significantly different between XY and ZW sex determination systems. The latter result remains qualitatively unchanged by including body size in the statistical model (Table 1: models 3 & 4, Figures 2 & 3b). Body size is not significantly related to sexual size dimorphism in species with GSD, although there is a trend for larger species showing more male-biased sexual size dimorphism (Table 1: model 4). By restricting the comparison of XY and ZW reptiles to 19 species that had ancestor with ESD as inferred by character reconstruction (Supplementary

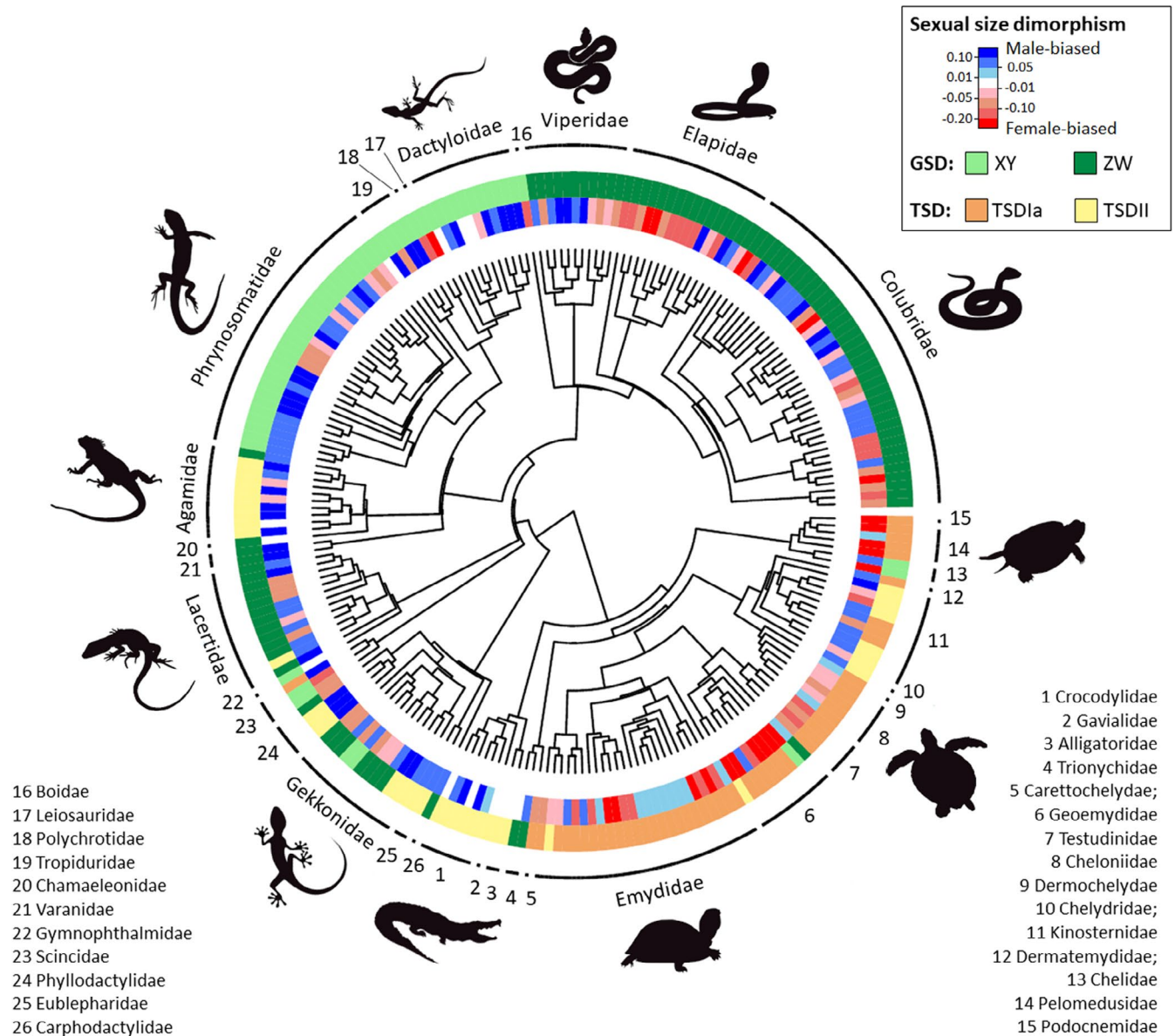


FIGURE 1 The phylogenetic distribution of sex determination systems (outer ring) and sexual size dimorphism (inner ring) in reptiles. Pictograms depict the major reptile clades ($N = 250$ species)

Material S2, Table S2), we again found no association between GSD type and sexual size dimorphism (Table 1: models 5, Figure 4a). This result remained nonsignificant after controlling for mean body size (Table 1: model 6).

However, this result might be influenced by outliers within the small sample; thus, we repeated this analysis by excluding one turtle and one chameleon species with male bias, and one turtle species with female bias in sexual size dimorphism. Without the outliers the association remained nonsignificant (Table 1: model 7; Figure 4b). However, when we control for mean body size in this smaller subset of GSD species, XY species have more male-biased sexual size dimorphism than ZW species (Table 1: model 8), with larger species also showing more male-biased sexual size dimorphism.

4 | DISCUSSION

Our study provides the most comprehensive phylogenetically controlled comparative assessment of the relationship between sex determination systems and sexual size dimorphism in nonavian reptiles. Using the largest dataset on sex determination in reptiles, our study demonstrates that the evolution of sex determination is associated with sexual size dimorphism. Although we did not directly investigate causal relations, this evolutionary correlation suggests that selection for sexual size dimorphism may be a driving force of evolutionary transitions between different types of sex determination in reptiles and thereby an important determinant of the diversity of sex determination in extant reptiles. Below we discuss the key results of our study.

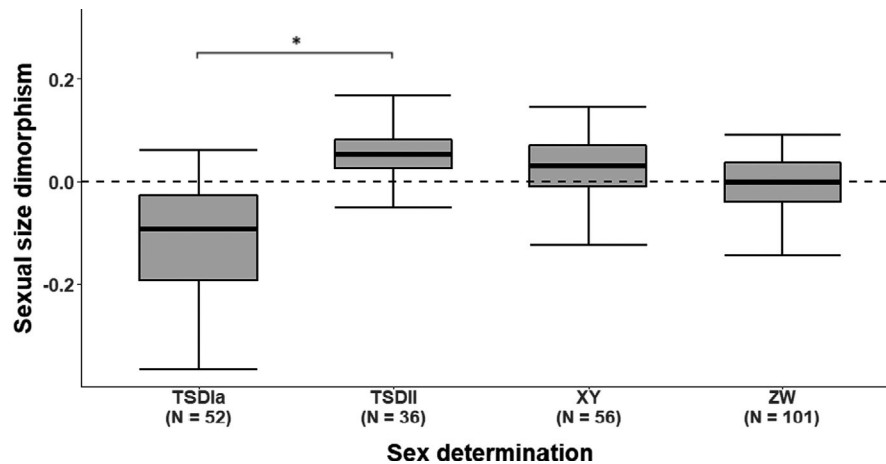


FIGURE 2 Sexual size dimorphism in relation to sex determination types in reptiles. Sexual size dimorphism was calculated as \log_{10} (male body length/female body length). Dashed horizontal line indicates monomorphism (i.e. no size difference between males and females), negative values correspond to female-biased dimorphism, and positive values correspond to male-biased dimorphism. Environmental sex determination (ESD) systems: TSDIa, TSDII. Genetic sex determination (GSD) systems: XY, ZW. N shows the number of species, and asterisk indicates significant difference; see statistics in Table 1. In each box plot, the thick middle line, box and whiskers represent the median, interquartile range and data range, respectively

First, the extent of sexual size dimorphism is more variable amongst ESD species than amongst GSD species. According to the “survival to maturity” hypothesis, ESD is more likely to evolve in species where males and females differ in their maturation age, because it allows the later-maturing sex to have higher annual survival rate in the juvenile life phase and thereby to reach sexual maturity (Bókonyi et al. 2019; Schwanz et al. 2016). Our result is consistent with this hypothesis as well as with a recent empirical study (Bókonyi et al. 2019) that, using a partially different dataset, found greater variance in the difference between male and female maturation age amongst TSD species than amongst GSD reptiles. The difference between sexes in maturation age is likely to be linked to sexual size dimorphism, since in indeterminately growing species such as reptiles, the later-maturing sex is expected to grow larger (Monnet et al. 2002). However, average life span does not seem to have a general effect on the variance of sexual size dimorphism, as the long-living crocodiles have significantly lower variance than both the long-living turtles and the short-living snakes and lizards.

Second, consistently with previous analyses using smaller sample sizes (Ewert & Nelson, 1991; Viets et al. 1994) we found that, on average, TSDIa species tend to have larger females than TSDII species. In turtles, the only group where both types of ESD are present, the association is no longer significant; however, the direction of the difference remains. Our result is in line with the theory that TSD is adaptive because it allows the individual to develop into the sex that has the higher fitness prospect under the prevailing environmental conditions (Charnov & Bull, 1977; Warner & Shine, 2008), either by growing faster and reaching the minimum size required for sexual maturity earlier (“temperature-dependent maturation”; Warner & Shine, 2008), or by growing larger and having higher fecundity (“temperature-dependent fertility”; Janzen & Paukstis, 1991a,b). To evaluate the contribution of these

life-history variables to the observed patterns, further studies are needed using fecundity and maturation data. However, it seems plausible that, when size difference between sexes is selected for, ESD can be adaptive.

Third, we identified a handful of reptile species with GSD which were inferred to evolve from an ESD ancestor, and the identification of these species are in line with previous ancestral state reconstructions (Pokorná & Kratochvíl, 2008; Gamble et al. 2015, and Sabath et al. 2016). This small subset of species shows a trend for more male-biased dimorphism in XY than in ZW systems, but this trend was statistically significant only after removing three outliers and controlling for mean body size (Figure 4). This result may change with ancestral state reconstructions using alternative phylogenies or larger sample sizes. Thus, our results neither support nor unequivocally confute the prediction of Adkins-Regan and Reeve (2014) in regard to the predicted association between sexual size dimorphism and GSD type in species with an ESD ancestry. A potential difficulty with testing the prediction is that once an XY or ZW system is evolved, the new sex determination system can influence the evolution of sexual size dimorphism, which may change the relationship between GSD and sexual size dimorphism after the initial evolution from ESD. For example, Reeve & Pfennig (2003) found that male secondary sexual characters – which are often associated with larger male body size – are more developed in ZW systems, supporting their assumption that genes driving these characters are better protected against random loss by genetic drift in species with male homogamety than in species with male heterogamety. Hence, selection for sexually dimorphic traits (including large size) can be more efficient in ZW than in XY systems. Even if an initial selection for large females caused a transition to ZW (as proposed by Kraak and de Loose 1994 and Adkins-Regan & Reeve, 2014), male-biased dimorphism may evolve afterwards because the new GSD system provides ideal conditions for intense

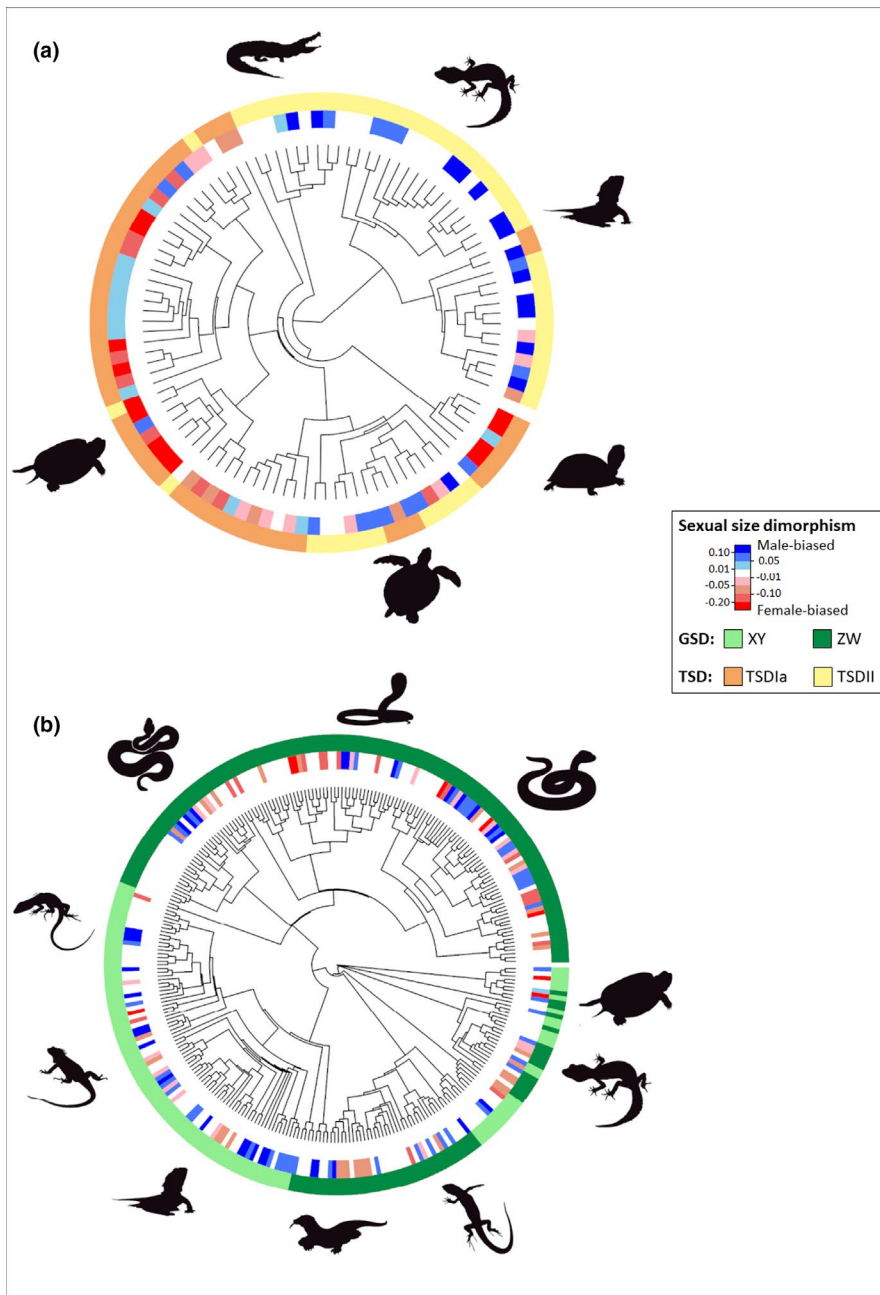


FIGURE 3 Phylogenetic distribution of sex determination systems (inner band) and sexual size dimorphism (outer band) in reptiles with (a) environmental sex determination (TSDIa and TSDII), and (b) with genetic sex determination (XY, ZW, see statistics in Table 1)

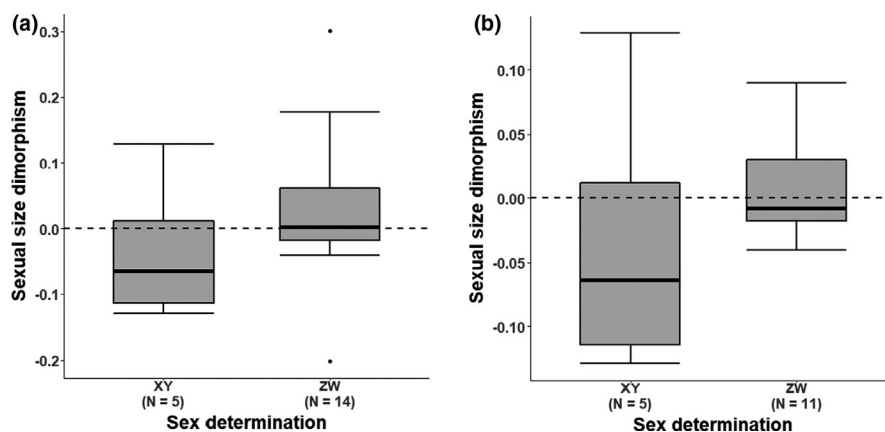


FIGURE 4 Sexual size dimorphism in relation to the type of genetic sex determination in reptiles with ESD ancestry. (a) N = 19 species, (b) three outlier species are excluded, N = 16 species. Sexual size dimorphism was calculated as $\log_{10}(\text{male body length} / \text{female body length})$. Dashed horizontal line indicates sexual monomorphism, negative values mean female-biased dimorphism and positive values correspond to male-biased (see statistics in Table 1)

sexual selection. In this sense, sexual size dimorphism can be both a cause and a consequence of GSD, and the direction and extent of dimorphism is dependent on specific selection processes in a given breeding system.

An important implication of our work is that sex determination may have knock-on effects on life histories, ecology and social behaviour of vertebrates, via its coevolution with sexual size dimorphism. Sexual size dimorphism is linked to several aspects of reproduction, for instance larger female body size often increases fecundity in ectothermic vertebrates (Han et al. 2013; Monroe et al. 2015; Olsson et al. 2002), whilst larger males are often more successful in intrasexual competition for mates (Cox et al. 2008; Shine, 1978; Vági & Hettyey, 2016). Whilst the type of ESD is associated with the direction of sexual dimorphism, GSD types relate to adult sex ratios, which tend to be biased towards the homogametic sex (Janzen & Paukstis, 1991a,b; Pipoly et al. 2015). Recent studies suggest that adult sex ratios are coupled with sexual selection and frequency dependent behaviours including courtships, mating systems and parental care (Liker et al. 2013, 2014). Thus, we believe that a productive future research direction is investigating the link between breeding systems and sex determination – as these processes of sexual selection provide feedback mechanisms to the evolution of sex determination beyond the proximate (genetic and developmental) causes.

In conclusion, our study finds that ESD species exhibit more variable sexual size dimorphism than GSD ones. We also show that TSD types predict the extent of sexual dimorphism. A possible future direction is the reconstruction of the ancestral states of sexual size dimorphism and the evolutionary transition rates between sex determination types, although the estimation of evolutionary transitions using extant species is controversial (Louca & Pennel, 2020). Nonetheless, understanding the implications of sex determination is urgent, since ectothermic vertebrates are facing new challenges stemming from climate change and increased anthropogenic effects including sex ratio-distorting pollutants (Bókony et al. 2017; Guillelte & Edwards, 2008; Jensen et al. 2018; Mizoguchi & Valenzuela, 2016; Schwanz et al. 2008). Therefore, the evolutionary and human-induced processes shaping sex determination will likely remain a hot topic in evolutionary ecology and beyond.

ACKNOWLEDGMENTS

The study was supported by the National Research, Development and Innovation Office of Hungary (grants PD 132819 to BV; K 116310 and ÉLVONAL KKP-126949 to TS and K-115402 to VB). On behalf of Project 'TetraClim', we thank for the usage of ELKH Cloud (<https://science-cloud.hu/>) that significantly helped us achieving the results published in this paper. We thank the two anonymous reviewers whose comments helped improve this manuscript.

CONFLICT OF INTERESTS

The authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

TS, BV and VB conceived the study. BV, ZV, RPF, AL, VB and TS designed the analyses. GK and VB collected data. GK and ZV conducted analyses. All authors wrote the paper.

Peer Review

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13774>.

DATA AVAILABILITY STATEMENT

All relevant data are within the paper and its electronic supplementary material and are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.z08kprc4>.

ORCID

Gergely Katona  <https://orcid.org/0000-0002-2889-7396>
 Balázs Vági  <https://orcid.org/0000-0002-0469-6784>
 Zolt Végvári  <https://orcid.org/0000-0002-2804-9282>
 András Liker  <https://orcid.org/0000-0001-8545-4869>
 Robert P. Freckleton  <https://orcid.org/0000-0002-8338-864X>
 Veronika Bókony  <https://orcid.org/0000-0002-2136-5346>
 Tamás Székely  <https://orcid.org/0000-0003-2093-0056>

REFERENCES

- Adkins-Regan, E., & Reeve, H. K. (2014). Sexual dimorphism in body size and the origin of sex-determination systems. *The American Naturalist*, 183, 519–536. <https://doi.org/10.1086/675303>
- Bachtrog, D., Mank, J. E., Peichel, C. L., Kirkpatrick, M., Otto, S. P., Ashman, T.-L., Hahn, M. W., Kitano, J., Mayrose, I., Ming, R., Perrin, N., Ross, L., Valenzuela, N., & Vamori, J. C., The Tree of Sex Consortium (2014). Sex determination: Why so many ways of doing it? *PLoS Biology*, 12, 7. <https://doi.org/10.1371/journal.pbio.1001899>
- Beukeboom, L. W., & Perrin, N. (2014). *The evolution of sex determination*. Oxford University Press.
- Bókony, V., Kövér, S. Z., Nemesházi, E., Liker, A., & Székely, T. (2017). Climate-driven shifts in adult sex ratios via sex reversals: The type of sex determination matters. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372, 20160325. <https://doi.org/10.1098/rstb.2016.0325>
- Bókony, V., Milne, G., Pipoly, I., Székely, T., & Liker, A. (2019). Sex ratios and bimaturism differ between temperature-dependent and genetic sex-determination systems in reptiles. *BMC Evolutionary Biology*, 19, 57. <https://doi.org/10.1186/s12862-019-1386-3>
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24, 127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Charnov, E. L., & Bull, J. (1977). When is sex environmentally determined? *Nature*, 266, 828–830. <https://doi.org/10.1038/266828a0>
- Cornejo-Páramo, P., Lira-Noriega, A., Ramírez-Suástegui, C., Méndez-de-la-Cruz, F. R., Székely, T., Urrutia, A. O., & Cortez, D. (2020). Sex determination systems in reptiles are related to ambient temperature but not to the level of climatic fluctuation. *BMC Evolutionary Biology*, 20, 103. <https://doi.org/10.1186/s12862-020-01671-y>
- Cox, R. M., Butler, M. A., & John-Alder, H. B. (2008). *The evolution of sexual size dimorphism in reptiles. Chapter 4 in Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. Oxford Biology.

- Ellegren, H. (2000). Evolution of the avian sex chromosomes and their role in sex determination. *Trends in Ecology & Evolution*, 15, 188–192. [https://doi.org/10.1016/S0169-5347\(00\)01821-8](https://doi.org/10.1016/S0169-5347(00)01821-8)
- Ewert, M. A., Jackson, D. R., & Nelson, C. E. (1994). Patterns of temperature-dependent sex determination in turtles. *Journal of Experimental Zoology*, 270, 3–15. <https://doi.org/10.1002/jez.1402700103>
- Ewert, M. A., & Nelson, C. E. (1991). Sex determination in turtles: Diverse patterns and some possible adaptive values. *Copeia*, 1991(1), 50–69. <https://doi.org/10.2307/1446248>
- Fairbairn, D., Blanckenhorn, W., Székely, T. (eds). (2007). *Sex, size and gender roles. Evolutionary studies of sexual size dimorphism* (pp. 1–266). Oxford University Press.
- Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *The American Naturalist*, 160, 712–726. <https://doi.org/10.1086/343873>
- Gamble, T., Coryell, J., Lynch, J., Scantlebury, D. P., & Zarkower, D. (2015). Restriction site-associated DNA sequencing (RAD-seq) reveals an extraordinary number of transitions among gecko sex-determining systems. *Molecular Biology and Evolution*, 32, 1296–1309. <https://doi.org/10.1093/molbev/msv023>
- Guillette, L. J. Jr, & Edwards, T. M. (2008). Environmental influences on fertility: Can we learn lessons from studies of wildlife? *Fertility and Sterility*, 89, 21–24. <https://doi.org/10.1016/j.fertnstert.2007.12.019>
- Han, X., & Fu, J. (2013). Does life history shape sexual size dimorphism in anurans? A comparative analysis. *BMC Evolutionary Biology*, 13, 27. <https://doi.org/10.1186/1471-2148-13-27>
- Janzen, F. J., & Paukstis, G. L. (1991a). A Preliminary test of the adaptive significance of environmental sex determination in reptiles. *Evolution*, 45, 435–440. <https://doi.org/10.2307/2409677>
- Janzen, F. J., & Paukstis, G. L. (1991b). Environmental sex determination in reptiles: Ecology, evolution, and experimental design. *The Quarterly Review of Biology*, 66, 149–179. <https://doi.org/10.1086/417143>
- Jensen, M. P., Camryn, A. D., Eguchi, T., Bell, I. P., LaCasella, E. L., Hilton, W. A., Hof, C. A. M., & Dutton, P. H. (2018). Environmental warming and feminization of one of the largest sea turtle populations on the world. *Current Biology*, 28, 154–159. <https://doi.org/10.1016/j.cub.2017.11.057>
- Kraak, S. B. M., & de Looze, E. M. A. (1994). A new hypothesis on the evolution of sex determination in vertebrates; Big females ZW, big males XY. *Netherlands Journal of Zoology*, 43, 260–273. <https://doi.org/10.1163/156854293X00034>
- Kumar, S., Stecher, G., Suleski, M., & Hedges, S. B. (2017). TimeTree: A resource for timelines, timetrees, and divergence times. *Molecular Biology and Evolution*, 34(7), 1812–1819. <https://doi.org/10.1093/molbev/msx116>
- Liker, A., Freckleton, R. P., & Székely, T. (2013). The evolution of sex roles in birds is related to adult sex ratio. *Nature Communications*, 4, 1587. <https://doi.org/10.1038/ncomms2600>
- Liker, A., Freckleton, R. P., & Székely, T. (2014). Divorce and infidelity are associated with skewed adult sex ratios in birds. *Current Biology*, 24, 880–884. <https://doi.org/10.1016/j.cub.2014.02.059>
- Louca, S., & Pennell, M. W. (2020). Extant timetrees are consistent with a myriad of diversification histories. *Nature*, 580, 502–505. <https://doi.org/10.1038/s41586-020-21761>
- Mizoguchi, B. A., & Valenzuela, N. (2016). Ecotoxicological perspectives of sex determination. *Sexual Development*, 10, 45–57. <https://doi.org/10.1159/000444770>
- Monnet, J. M., & Cherry, M. I. (2002). Sexual size dimorphism in anurans. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 269, 2301–2307. <https://doi.org/10.1098/rspb.2002.2170>
- Monroe, M. J., South, S. H., & Alonzo, S. H. (2015). The evolution of female fecundity is associated with female body size but not female-biased sexual size dimorphism in frogs. *Journal of Evolutionary Biology*, 28, 1793–1803. <https://doi.org/10.1111/jeb.12695>
- Olsson, M., Shine, R., Wapstra, E., Ujvari, B., & Madsen, T. (2002). Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. *Evolution*, 56, 1538–1542. <https://doi.org/10.1111/j.0014-3820.2002.tb01464.x>
- Organ, C. L., Janes, D. E., Meade, A., & Pagel, M. (2009). Genotypic sex determination enabled adaptive radiations of extinct marine reptiles. *Nature*, 461, 389–392. <https://doi.org/10.1038/nature08523>
- Orme, D., & Freckleton, R. P. (2013). *The caper package: Comparative analysis of phylogenetics and evolution in R*. R package version, 5(2) <https://cran.r-project.org/web/packages/caper/vignettes/caper.pdf>
- Pen, I., Uller, T., Feldmeyer, B., Harts, A., While, G. M., & Wapstra, E. (2010). Climate-driven population divergence in sex-determining systems. *Nature*, 468, 436–438. <https://doi.org/10.1038/nature09512>
- Pincheira-Donoso, D., & Hunt, J. (2017). Fecundity selection theory: Concepts and evidence. *Biological Reviews*, 92, 341–356. <https://doi.org/10.1111/brv.12232>
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team (2020). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-143. <https://CRAN.R-project.org/package=nlme>
- Pipoly, I., Bókony, V., Kirkpatrick, M., Donald, P. F., Székely, T., & Liker, A. (2015). The genetic sex-determination system predicts adult sex ratios in tetrapods. *Nature*, 527, 91–94. <https://doi.org/10.1038/nature15380>
- Pokorná, M., & Kratochvil, L. (2008). Phylogeny of sex-determining mechanisms in squamate reptiles: Are sex chromosomes an evolutionary trap? *Zoological Journal of the Linnean Society*, 156, 168–183. <https://doi.org/10.1111/j.1096-3642.2008.00481.x>
- Pokorná, M., & Kratochvil, L. (2016). What was the ancestral sex-determining mechanism in amniote vertebrates? *Biological Reviews*, 91, 1–12. <https://doi.org/10.1111/brv.12156>
- Pyron, R. A., Burbrink, F. T., & Wiens, J. J. (2013). A phylogeny and revisited classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology*, 13, 93. <https://doi.org/10.1186/1471-2148-13-93>
- R-Core-Team R (2013). *A language and environment for statistical computing*. Available from: <http://www.r-project.org/>
- Reeve H. K., & Pfennig D. W. (2003). Genetic biases for showy males: Are some genetic systems especially conducive to sexual selection? *Proceedings of the National Academy of Sciences*, 100, 1089–1094. <http://dx.doi.org/10.1073/pnas.0337427100>
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>
- Rohner, P. T., Blanckenhorn, W. U., & Puniamoorthy, N. (2016). Sexual selection on male size drives the evolution of male-biased sexual size dimorphism via the prolongation of male development. *Evolution*, 70, 1189–1199. <https://doi.org/10.1111/evo.12944>
- Sabath, N., Itescu, Y., Feldman, A., Meiri, S., Mayrose, I., & Valenzuela, N. (2016). Sex determination, longevity, and the birth and death of reptilian species. *Ecology and Evolution*, 6, 5207–5220. <https://doi.org/10.1002/ece3.2277>
- Scharlt, M. (2004). Sex chromosome evolution in non-mammalian vertebrates. *Current Opinion in Genetics & Development*, 14, 634–641. <https://doi.org/10.1016/j.gde.2004.09.005>
- Schwanz, L. E., Cordero, G. A., Charnov, E. L., & Janzen, F. J. (2016). Sex-specific survival to maturity and the evolution of environmental sex determination. *Evolution*, 70, 329–341. <https://doi.org/10.1111/evo.12856>
- Schwanz, L. E., & Janzen, F. J. (2008). Climate change and temperature-dependent sex determination: Can individual plasticity in nesting phenology prevent extreme sex ratios? *Physiological and Biochemical Zoology*, 81, 826–834. <https://doi.org/10.1086/590220>
- Serrano-Meneses, M. A., & Székely, T. (2006). Sexual size dimorphism in seabirds: Sexual selection, fecundity selection and differential niche-utilisation. *Oikos*, 113, 385–394. <https://doi.org/10.1111/j.0030-1299.2006.14246.x>

- Shine, R. (1978). Sexual size dimorphism and male combat in snakes. *Oecologia*, 33, 269–277. <https://doi.org/10.1007/BF00348113>
- Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 8, 1639–1644. <https://doi.org/10.1111/2041-210X.12797>
- Székely, T., Freckleton, R. P., & Reynolds, J. D. (2004). Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *PNAS*, 33, 12224–12227. <https://doi.org/10.1073/pnas.0404503101>
- The Tree of Sex Consortium, Ashman, T.-L., Bachtrog, D., Blackmon, H., Goldberg, E. E., Hahn, M. W., Kirkpatrick, M., Kitano, J., Mank, J. E., Mayrose, I., Ming, R., Otto, S. P., Peichel, C. L., Pennell, M. W., Perrin, N., Ross, L., Valenzuela, N., & Vamosi, J. C. (2014). Tree of sex: A database of sexual systems. *Scientific Data*, 1, 140015. <https://doi.org/10.1038/sdata.2014.15>
- Vági, B., & Hettyey, A. (2016). Intraspecific and interspecific competition for mates: *Rana temporaria* males are effective satyrs of *Rana dalmatina* females. *Behavioral Ecology and Sociobiology*, 70, 1477–1484. <https://doi.org/10.1007/s00265-016-2156-5>
- Valenzuela, N., & Lance, V. A. (2004). *Temperature-dependent sex determination in vertebrates*. Smithsonian InstitutionBook. <https://doi.org/10.5479/si.9781944466213>
- Viets, B. E., Ewert, M. A., Talent, L. G., & Nelson, C. E. (1994). Sex-determining mechanism in squamate reptiles. *Journal of Experimental Zoology*, 270, 45–56. <https://doi.org/10.1002/jez.1402700106>
- Warner, D. A., & Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile. *Nature*, 451, 566–568. <https://doi.org/10.1038/nature06519>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Katona G, Vági B, Végvári Z, et al. Are evolutionary transitions in sexual size dimorphism related to sex determination in reptiles?. *J Evol Biol*. 2021;34:594–603. <https://doi.org/10.1111/jeb.13774>