doi:10.1111/evo.14410

Sex-related differences in aging rate are associated with sex chromosome system in amphibians

Hugo Cayuela,[1,2] Jean-François Lemaître,[3] Jean-Paul Léna,[4] Victor Ronget,[5] Iñigo Martínez-Solano,[6] Erin Muths,[7] David S. Pilliod,[8] Benedikt R. Schmidt,[9,10] Gregorio Sánchez-Montes,[6] Jorge Gutiérrez-Rodríguez,[6,11] Graham Pyke,[12,13] Kurt Grossenbacher,[14] Omar Lenzi,[9] Jaime Bosch,[6,15,16] Karen H. Beard,[17] Lawrence L. Woolbright,[18] Brad A. Lambert,[19] David M. Green,[20] Nathalie Jreidini,[20] Justin M. Garwood,[21] Robert N. Fisher,[22] Kathleen Matthews,[23] David Dudgeon,[24] Anthony Lau,[25] Jeroen Speybroeck,[26] Rebecca Homan,[27] Robert Jehle,[28] Eyup Başkale,[29] Emiliano Mori,[30] Jan W. Arntzen,[31] Pierre Joly,[4] Rochelle M. Stiles,[32] Michael J. Lannoo,[33] John C. Maerz,[34] Winsor H. Lowe,[35] Andrés Valenzuela-Sánchez,[36,37] Ditte G. Christiansen,[9] Claudio Angelini,[38] Jean-Marc Thirion,[39] Juha Merilä,[24,40] Guarino R. Colli,[41] Mariana M. Vasconcellos,[42] Taissa C. V. Boas,[41] Ísis da C. Arantes,[43] Pauline Levionnois,[4] Beth A. Reinke,[44] Cristina Vieira,[3] Gabriel A. B. Marais,[3,45] Jean-Michel Gaillard,[3] and David A. W. Miller[46]

[1]*Department of Ecology and Evolution, University of Lausanne, Lausanne 1015, Switzerland*

[2]*E-mail: hugo.cayuela51@gmail.com*

[3]*Laboratoire de Biométrie et Biologie Evolutive, Université Lyon 1, CNRS, UMR 5558, Villeurbanne F-769622, France*

[4]*Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR5023 LEHNA, Villeurbanne F-69622, France*

[5]*Unité Eco-anthropologie (EA), Muséum National d'Histoire Naturelle, CNRS, Université Paris Diderot, Paris F-75016, France*

[6]*Museo Nacional de Ciencias Naturales, CSIC, Madrid 28006, Spain*

[7]*U.S. Geological Survey, Fort Collins Science Center, Fort Collins, Colorado 80526*

[8]*U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Boise, Idaho 83706*

[9]*Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zürich 8057, Switzerland*

[10]*Info fauna karch, Neuchâtel 2000, Switzerland*

[11]*Department of Integrative Ecology, Estación Biológica de Doñana (EBD-CSIC), Seville, Spain*

[12]*Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P. R. China*

[13]*Department of Biological Sciences, Macquarie University, Ryde, NSW 2109, Australia*

[14]*Abteilung Wirbeltiere, Naturhistorisches Museum, Bern 3005, Switzerland*

[15]*UMIB-Research Unit of Biodiversity (CSIC, UO, PA), Universidad de Oviedo, Campus de Mieres, Mieres 33600, Spain*

[16]*Centro de Investigación, Seguimiento y Evaluación, Sierra de Guadarrama National Park, Rascafría 28740, Spain*

[17]*Department of Wildland Resources and the Ecology Center, Utah State University, Logan, Utah 84322*

[18]*Biology Department, Siena College, Loudonville, New York 12211*

[19]*Colorado Natural Heritage Program, Colorado State University, Fort Collins, Colorado 80523-1475*

[20]*Redpath Museum, McGill University, Montreal, QC H3A 0C4, Canada*

[21]*California Department of Fish and Wildlife, Arcata, California 95521*

[22]*Western Ecological Research Center, U.S. Geological Survey, San Diego, California 92101*

[23]*USDA Forest Service, Pacific Southwest Research Station, Albany, California*
[24]*Division of Ecology and Biodiversity, School of Biological Sciences, The University of Hong Kong, Hong Kong SAR*
[25]*Science Unit, Lingnan University, Hong Kong, China*
[26]*Research Institute for Nature and Forest, Brussel 1000, Belgium*
[27]*Biology Department, Denison University, Granville, Ohio*
[28]*School of Science, Engineering and Environment, University of Salford, Salford, UK*
[29]*Department of Biology, Faculty of Science and Arts, Pamukkale University, Denizli, Turkey*
[30]*Consiglio Nazionale delle Ricerche, Istituto di Ricerca sugli Ecosistemi Terrestri, Sesto Fiorentino 50019, Italy*
[31]*Naturalis Biodiversity Center, Leiden, The Netherlands*
[32]*San Francisco Zoological Society, San Francisco, California 94132*
[33]*Indiana University School of Medicine–TH, Terre Haute, Indiana 47809*
[34]*Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia*
[35]*Division of Biological Sciences, University of Montana, Missoula, Montana 59812*
[36]*Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Valdivia 5090000, Chile*
[37]*ONG Ranita de Darwin, Valdivia 5112144, Chile*
[38]*Salamandrina Sezzese Search Society, Sezze 04018, Italy*
[39]*Objectifs Biodiversité, Pont-l'Abbé-d'Arnoult 17250, France*
[40]*Ecological Genetics Research Unit, Research Programme in Organismal and Evolutionary Biology, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki 00014, Finland*
[41]*Departamento de Zoologia, Universidade de Brasília, Brasília 70910-900, Brazil*
[42]*Department of Biology, City College of New York, The City University of New York, New York, New York 10031*
[43]*Department of Biology, University of Mississippi, Oxford, Mississippi 38677*
[44]*Department of Biology, Northeastern Illinois University, Chicago, Illinois 60625*
[45]*LEAF- Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal*
[46]*Department of Ecosystem Sciences and Management, The Pennsylvania State University, University Park, Pennsylvania*

Received July 23, 2021
Accepted November 16, 2021

Sex-related differences in mortality are widespread in the animal kingdom. Although studies have shown that sex determination systems might drive lifespan evolution, sex chromosome influence on aging rates have not been investigated so far, likely due to an apparent lack of demographic data from clades including both XY (with heterogametic males) and ZW (heterogametic females) systems. Taking advantage of a unique collection of capture–recapture datasets in amphibians, a vertebrate group where XY and ZW systems have repeatedly evolved over the past 200 million years, we examined whether sex heterogamy can predict sex differences in aging rates and lifespans. We showed that the strength and direction of sex differences in aging rates (and not lifespan) differ between XY and ZW systems. Sex-specific variation in aging rates was moderate within each system, but aging rates tended to be consistently higher in the heterogametic sex. This led to small but detectable effects of sex chromosome system on sex differences in aging rates in our models. Although preliminary, our results suggest that exposed recessive deleterious mutations on the X/Z chromosome (the "unguarded X/Z effect") or repeat-rich Y/W chromosome (the "toxic Y/W effect") could accelerate aging in the heterogametic sex in some vertebrate clades.

KEY WORDS: **Aging, senescence, sex chromosome, amphibians.**

Sex-related differences in mortality are widespread across the tree of life (Austad and Fischer 2016; Marais et al. 2018). This finding, however, strongly relies on comparative analyses of sex differences in lifespan in mammals and birds (Promislow et al. 1992; Clutton-Brock and Isvaran 2007; Maklakov and Lummaa 2013; Tidière et al. 2015). Among mammals, females typically outlive males, with an adult lifespan that is on average 18.5% longer (Lemaître et al. 2020a). The picture is less clear in birds, but the evidence suggests that males tend to have higher adult survival rates than females (Liker and Székely 2005). Sex differences in lifespan are often interpreted in terms of mortality aging (also known as actuarial senescence), which is usually assessed by the rate of increase in age-specific mortality (i.e., aging rate) (e.g., Lemaître et al. 2020a). However, the relationship between aging rate and lifespan is far from straightforward (Kowald 2002). In fact, these two metrics of mortality appear to be largely uncoupled (Péron et al. 2019), which explains why, despite a consistently longer lifespan for females, there are no consistent sex differences in aging rates across mammals (Lemaître et al. 2020a). Current research on aging emphasizes the need for studies that investigate the evolutionary roots of sex differences in mortality, as well as their underlying mechanisms, while considering both lifespan and aging metrics (Lemaître et al. 2020b; Ronget and Gaillard 2020).

Among the wide range of factors that have been proposed to mitigate the direction and the magnitude of sex differences in lifespan or rate of aging, the role of sex chromosomes is gaining recognition (Marais et al. 2018) even though the underlying genetic mechanisms have not yet been fully understood. In mammals and birds, the homogametic sex (XX females and ZZ males, respectively) shows a higher survival rate than the heterogametic sex (XY males and ZW females, respectively), which fits the general picture of longer lived mammalian females and avian males (Liker and Székely 2005; Lemaître et al. 2020a). However, birds and mammals differ in many respects which, in the absence of fine-scale genetic data, make it impossible to draw any firm conclusion about the role of sex chromosomes in shaping sexual differences in mortality patterns (Maklakov and Lummaa 2013).

Other vertebrates, such as amphibians and reptiles, have repeatedly transitioned between XX/XY and ZW/ZZ systems during their evolutionary history (Bachtrog et al. 2014; Pennell et al. 2018). These taxa therefore offer a unique opportunity to assess the influence of sex chromosomes on mortality patterns. Taking advantage of this diversity in sex-chromosome systems, Pipoly et al. (2015) observed that the adult sex ratio in reptiles and amphibians was consistently biased toward males in species with the ZW system, and toward females in species with the XY system, suggesting that the heterogametic sex suffers from higher adult mortality. In addition, the survival advantage of the homogametic sex has been examined recently in a large-scale comparative analysis—encompassing both vertebrates and invertebrates—which revealed that a given measure of lifespan (mostly mean lifespan) is indeed generally longer for the homogametic sex, irrespective of the sex chromosome system considered (i.e., XX/XY; ZZ/ZW) (Xirocostas et al. 2020).

Two mechanisms have been proposed to explain the association between sex-specific differences in longevity and the operating sex chromosome system (Marais et al. 2018). First, in the heterogametic sex, where the X/Z chromosome is hemizygous, all mutations—including the recessive deleterious ones—are expressed, which could accelerate aging (the "unguarded X/Z effect"; Trivers 1985). Second, the nonrecombining region of the Y/W chromosome typically harbors many transposable elements, which are normally silenced (Bachtrog 2013; Brown et al. 2020). However, reactivation of transposable elements late in life could accelerate aging of the heterogametic sex (the "toxic Y/W" effect; Marais et al. 2018; Brown et al. 2020), by increasing somatic mutations. Both these effects imply that sex chromosomes can affect aging. However, so far only the association between sex chromosome system and measures of longevity has been studied (Xirocostas et al. 2020), leaving the issue of a possible effect of sex chromosome system on aging rate unresolved.

To fill this knowledge gap, we compiled long-term capture–recapture datasets (times series with a total of 56,207 marked individuals) for 36 amphibian species to examine if and how the genetic sex-determination system is related to differences in aging rates between sexes. Amphibians are good candidates to address this issue because ZW and XY systems have switched during evolutionary history on multiple occasions during the past 200 million years (Hillis and Green 1990; Miura 2017; Pennell et al. 2018). We first analyzed the relationship between standard metrics of adult lifespan and aging rate (Lemaître et al. 2020b; Ronget and Gaillard 2020) while controlling for phylogenetic relatedness across species, and then investigated whether the heterogametic sex (XY males and ZW females) consistently experiences shorter lifespan or higher aging rates than the homogametic sex (XX females and ZZ males).

Materials and Methods

CAPTURE–RECAPTURE DATA AND SEX DETERMINATION

We gathered a unique collection of capture–recapture data of individuals from 36 amphibian species (25 anurans and 11 urodeles). These longitudinal data allow robust estimates of age-specific mortality (Ronget and Gaillard 2020). Our database encompasses 56,207 individuals (24,187 females and 32,020 males) that have been marked and surveyed during study periods

ranging from 6 to 33 years (mean: 12.90 ± 6.65). A description of the capture–recapture data (e.g., sample size per sex for each species, number of years of survey, percentage of known-age individuals, and survey period) is given in Supporting Information S2 and Table S1. The heterogametic sex of each species was identified using the Tree of Sex Database (Tree of Sex Consortium 2014), the amphibian karyotype database compiled by Perkins et al. (2019), and two additional recent studies (Jeffries et al. 2018; Dufresnes et al. 2020). As the sex-determination system was undescribed in 21 species but documented in closely related species, we used Bayesian stochastic mapping implemented in the R package phytools (Revell 2012) to infer the heterogametic sex of those species. For that purpose, we considered 110 amphibian species (Supporting Information S2 and Table S2) for which the genetic sex determination systems were reported in online databases (Fig. 2B).

ESTIMATION OF ADULT LIFESPAN AND RATE OF MORTALITY AGING

We used adult observations only to focus on the mortality that occurs after the age of first reproduction (as age-specific mortality is assumed not to increase before that age; Williams 1957; Hamilton 1966). Adult lifespan and mortality aging rate were then estimated using Bayesian survival trajectory analyses implemented in the R package BaSTA (Colchero and Clark 2012; Colchero et al. 2012). Simulations by Colchero and Clark (2012) showed that BaSTA models are highly efficient to investigate age-dependent mortality even when a substantial proportion of dates of birth and death are unknown and recapture probability is less than 1. BaSTA, therefore, allowed us to account for imperfect detection, left-truncated (i.e., unknown birth date (age)) and right-censored (i.e., unknown death date) capture–recapture data in our analysis. To be conservative, we specified time-dependent recapture probabilities for all species, allowing for annual differences in the proportion of individuals among those alive in the population that were observed. Four MCMC chains were run with 50,000 iterations and a burn-in of 5000. Chains were thinned by a factor of 50. Model convergence was evaluated using the diagnostic analyses implemented in BaSTA, which calculates the potential scale reduction for each parameter to assess convergence.

We fitted a Siler model on age-specific mortality data (Siler 1979) to obtain comparable metrics for each species (for a justification of the use of this model, see Lemaître et al. 2020a). The five-parameter Siler model is given by:

$$\mu(x) = a_0 \exp(-a_1 x) + c + b_0 \exp(b_1 x)$$

where a_0, a_1, b_0, b_1, and $c \geq 0$ are the parameters of the mortality function, x the age in years, and $\mu(x)$ the age-specific rate of mortality. The first exponential function with parameters a describes the changes in mortality in the early adult stage, whereas c gives the lower limit of mortality during the adult stage. The second exponential function with b parameters corresponds to the mortality increase during the senescent stage. The parameter b_1 of the Siler model measures the exponential increase in mortality rate with age and corresponds to the rate of mortality aging in vertebrates (Lemaître et al. 2020a; Ronget and Gaillard 2020). We estimated sex-specific median adult lifespan (in years), corresponding to the age at which 50% of the individuals alive at the onset of adulthood (i.e., when first reproduction occurs) are dead (i.e., when cumulative survivorship reaches 0.5), and adult lifespan at 80% (i.e., when cumulative survivorship reaches 0.2) from the life tables extracted from BaSTA models. The use of these two thresholds is based on the study of Lemaître et al. (2020a).

STATISTICAL ANALYSES

We investigated the overall sex difference in aging rate according to the species sex chromosome system using univariate phylogenetic mixed models embedded in a Bayesian framework and implemented in the MCMCglmm package (Hadfield 2010). Sex differences in aging rates were computed as the ratio between male and female trait value on a log scale (i.e., difference in aging rate $= \log(\frac{\text{male aging rate}}{\text{female aging rate}})$) and treated as the response variable. The sex chromosome system of the species was introduced as an explanatory factor. The effect of the phylogenetic relatedness among species was accommodated through a random effect based on a standardized phylogenetic variance–covariance matrix extracted from an amphibian phylogenetic tree. This tree was constructed by pruning the amphibian Tree of Life published by Jetz and Pyron (2018) to include only species targeted in our study by using the phylogeny subsets tool in https://vertlife.org/phylosubsets/. Ten thousand trees from the pseudo-posterior distribution of trees in Jetz and Pyron (2018) were downloaded and a maximum clade credibility consensus tree was constructed with TreeAnnotator v1.10.1 (distributed as part of the BEAST software package; Suchard et al. 2018) and used for downstream analyses. The variance decomposition method was then used to calculate the proportion of variance explained by the phylogenetic relatedness (hereafter referred to as H^2, equivalent to the Pagel's phylogenetic signal; Hadfield and Nakagawa 2010) in both sex-specific traits and between-sex differences in traits. Given the moderate sample size of our dataset (i.e., 36 species) and the reduced expected phylogenetic variance, we selected two sets of inverse Wishart priors, one weakly informative (i.e., nu = 0.2, V = 1) and the other more informative (i.e., nu = 1, V = 1). Sensitivity of the results to the priors was controlled using Gelman and Rubin's convergence diagnostic (Gelman and Rubin 1992) based on the calculation of the potential scale reduction factor (hereafter referred as *psrf*)

between Markov chains simulated under both priors. The estimates of the heterogametic sex effect were found to be insensitive to the prior (i.e., *psrf* < 1.02) but not so for the phylogenetic variance parameter (i.e., *psrf* = 1.3), as expected given our moderate sample size. For each parameter, we reported the mean of the highest posterior density distribution as well as the lower and upper limits of its 95% credible interval (hereafter referred to as 95% confidence interval [CI]).

To detail the relative effect of sex chromosome on aging rates within each sex, we also performed a bivariate analysis where the log-transformed aging rates of both sexes were treated as dependent variables. In this model, the aging rates of both sexes were thus considered as two evolving traits, allowing us to estimate their respective phylogenetic relatedness (and the phylogenetic correlation between sexes) as well as the relative displacement entailed by the heterogametic sex. To this aim, a multivariate phylogenetic variance matrix was built, and both a species random effect and a separate residual term for each sex were introduced in the model. As in the univariate analysis, the model was implemented in the MCMCglmm package, and two sets of inverse Wishart priors were used for the multivariate phylogenetic variance components (nu = 2, V = 0.5 and nu = 2, V = 0.2, respectively). Like in the univariate analysis, a higher sensitivity to the prior was found for the phylogenetic variance components (i.e., *psrf* < 1.01) compared to the heterogametic sex effect (i.e., *psrf* < 1.03).

We performed similar bivariate and multivariate analyses to examine the effect of sex chromosome system on median lifespan and lifespan 80% (the method used is described in detail in Supporting Information S3). Moreover, to examine the extent to which aging rates were associated with both median and 80% lifespan across species for a given sex, we performed a univariate analysis separately for each sex (see the description of the method in Supporting Information S2).

Results

CORRELATION BETWEEN LIFESPAN AND SENESCENCE RATE

Adult lifespan and aging rate of a given species were negatively correlated (lifespan 80%: slopes of −0.30 [95% CI: −0.47; −0.14] with $R^2 = 0.27$ and −0.29 [95% CI: −0.47; −0.11] with $R^2 = 0.22$ for males and females, respectively, see Supporting Information S2 and Fig. S1 for detailed results). We found similar relationships between median adult lifespan (i.e., age when 50% of the individuals alive at the age of first reproduction were dead) and aging rates (slope of −0.30 [95% CI: −0.46; −0.14] with $R^2 = 0.27$ and slope of −0.29 [95% CI: −0.47; −0.12] with R^2 =0.22, respectively, for males and females).

SEX HETEROGAMY AND AGING RATE

Both the direction and magnitude of sex differences in aging rates (measured as the rate of the exponential increase of mortality with age) varied considerably (Fig. 1), ranging from species where females show higher aging rates (e.g., 0.20 in females vs. 0.07 in males in the salamander *Pleurodeles waltl*) to species where aging rates are more pronounced in males (e.g., 1.19 in males vs. 0.52 in females in the frog *Pelophylax perezi*) (Fig. 2A). Fifty-eight percent of the species with a XX/XY system ($N = 27$) had a higher aging rate in males than in females, whereas 71% of the species with a ZZ/ZW system ($N = 9$) showed a higher aging rate in females than in males. The aging rate of XY males was 24% higher (median of the difference) than that of XX females. In contrast, ZW females had an aging rate 20% higher than ZZ males.

Bayesian univariate mixed models showed that the direction of sex differences in aging rates differed between XY and ZW systems (Fig. 2B) and indicated a weak phylogenetic signal in sex-specific variation in aging rates (H^2: 0.25 [95% CI: 0.04; 0.52]). The posterior distribution of the log-scaled ratio of aging rate between sexes always included zero, suggesting moderate sex-specific variation in aging rate within XY and ZW systems. Homogametic males tended to show lower aging rates (−0.54 [95% CI: −1.13; 0.09]) than heterogametic females in the ZW system. By contrast, in the XY system, heterogametic males displayed marginally higher aging rates than homogametic females (0.13 [95% CI: −0.33; 0.57]). Nonetheless, this consistent effect of sex heterogamy on sex differences in aging rates in XY and ZW systems led to small but detectable effect of sex chromosome system on sex-specific variation in aging rates. Our models thus indicated that the log-scaled ratio of aging rate was higher (0.60 [95% CI: 0.11;1.22]; Fig. 2C)—namely more biased toward males—in the XY system than in the ZW system where aging rates were female biased. Additional analyses excluding species with uncertain sex chromosome assignment or including the probability of sex chromosome assignment as the explanatory variable provided similar results (Supporting Information S4).

Bayesian bivariate mixed models also indicated a sex-specific effect of the sex chromosome system on the aging rate. In particular, we found that the aging rate tended to be higher in XY than in ZZ males (i.e., 0.93 [95% CI: −0.02; 1.95]; Fig. 2D), whereas only a very weak difference was detected in females (i.e., −0.30 [95% CI: −0.66; 1.22]; Fig. 2E). Finally, our findings suggest weak phylogenetic inertia for aging rate across amphibians, as indicated by the limited contribution of the phylogenetic variance to the aging rate of both sexes (H^2 for males and females: 0.22 [95% CI: 0.02; 0.61] and 0.24 [95% CI: 0.02; 0.66], respectively). There was no substantial difference of H^2 between sexes as indicated by the posterior distribution of its size effect (−0.01 [−0.27; 0.22]).

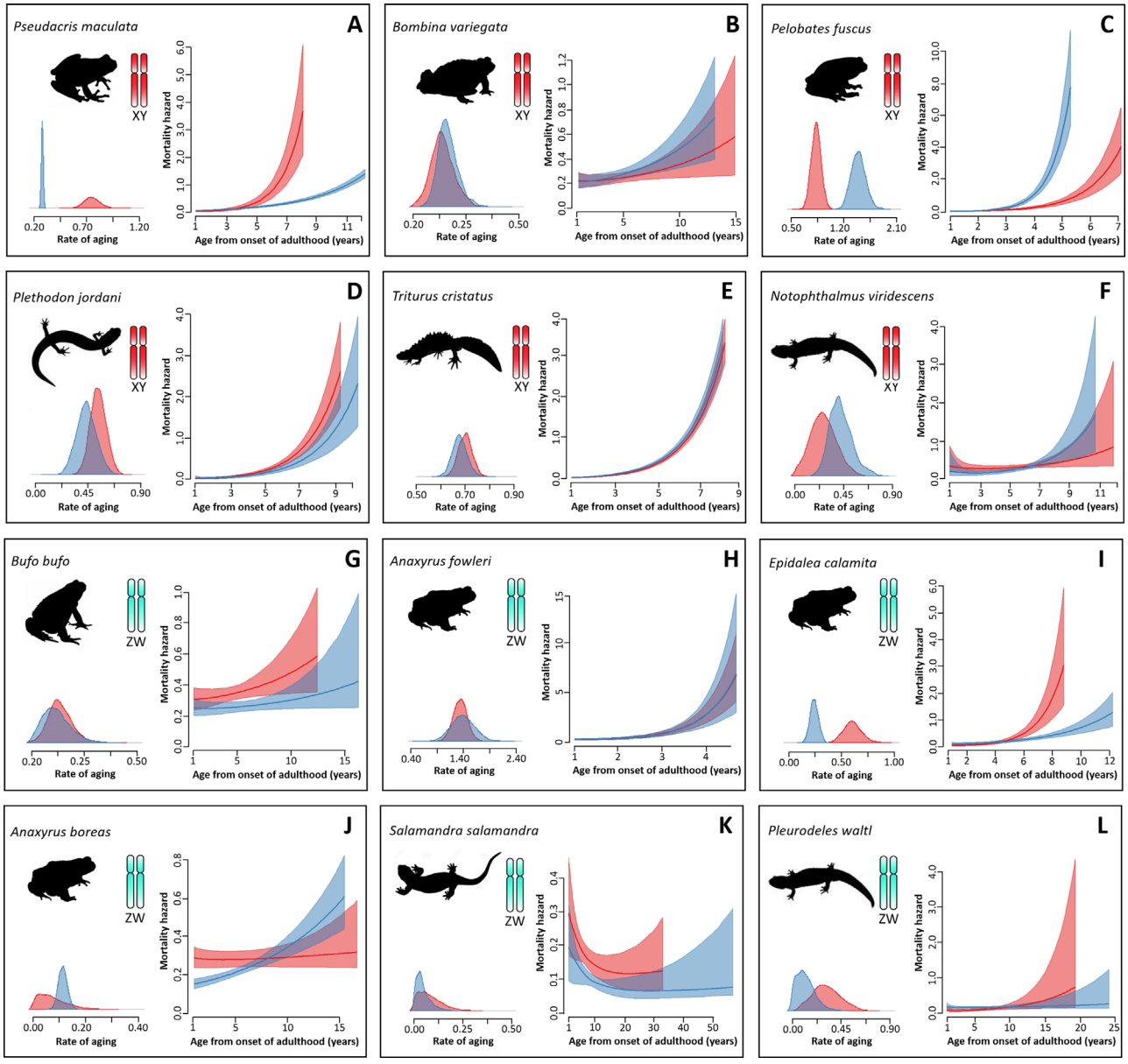

Figure 1. Sex-specific variation in aging patterns and rates in 12 randomly selected species of amphibians with XY (panels A–F) and ZW (panels G–L) sex chromosome systems. Mortality curves of males and females are shown in blue and red, respectively. Aging patterns of the other species are presented in Supporting Information Figs. S4–S11.

SEX HETEROGAMY AND LIFESPAN

Neither the lifespan 80% (the age when 80% of the individuals alive at the age of first reproduction were dead) nor the lifespan 50% were found to vary according to the sex chromosome system, as indicated by the posterior mean of the corresponding size effect (see Supporting Information S3 and Fig. S2): for lifespan 80%, the slope was −0.28 [95% CI: −0.90; 0.35] and −0.05 [95% CI: −0.69; 0.55] for males and females, respectively; for lifespan 50%, it was −0.22 [95% CI: −0.81; 0.35] and −0.08 [95% CI: −0.66; 0.54] for males and females, respectively. By contrast, our analysis revealed a substantial phylogenetic inertia on lifespan in both sexes. For lifespan 80%, H^2 was 0.56 [95% CI: 0.24; 0.87] and 0.54 [95% CI: 0.24; 0.86] for males and females, respectively, and there was no substantial difference of H^2 between both sexes as revealed by its posterior distribution (0.004 [95% CI:−0.2795; 0.2964]); for lifespan 50%, it was 0.53 and 0.53 for both males [95% CI: 0.26; 0.82] and females [95% CI:0.24; 0.81] and there was no evidence for a difference of H^2 between sexes as revealed by its posterior distribution (0.001 [95% CI:−0.3164; 0.3074]).

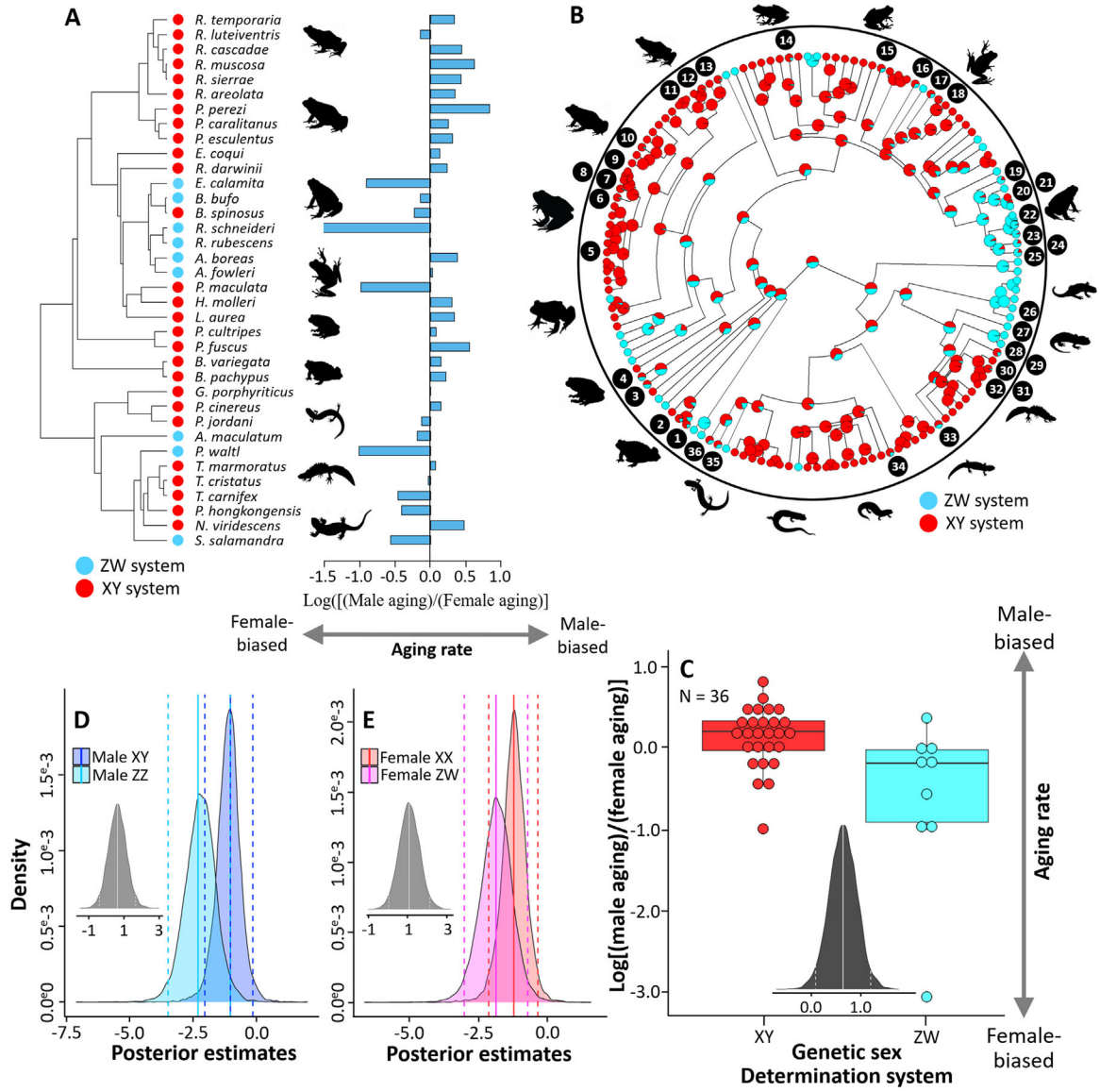

Figure 2. Sex chromosome system and sex-specific aging rate in 36 amphibian species. (A) Sex differences in aging rate across amphibians: for a given species, the sex difference was measured as the ratio [(male aging rate)/(female aging rate)]. (B) Bayesian stochastic mapping implemented in the R package phytools used to infer the sex chromosome system in species for which genetic sex-determination system was not documented (26 of 36 species)—Bayesian posterior probabilities for tips are provided in Supporting Information Table S2. We considered 111 amphibian species for which sex chromosome system documented in online databases (Tree of Sex Consortium 2014; Perkins et al. 2019) and recently published papers (Jeffries et al. 2018; Dufresnes et al. 2020). The 36 species considered in our study are mapped along the tree. 1: *Bombina pachypus*, 2: *Bombina variegata*, 3: *Pelobates cultripes*, 4: *Pelobates fuscus*, 5: *Rana temporaria*, 6: *Rana luteiventris*, 7: *Rana cascadae*, 8: *Rana muscosa*, 9: *Rana sierrae*, 10: *Rana areolata*, 11: *Pelophylax esculentus*, 12: *Pelophylax caralitanus*, 13: *Pelophylax perezi*, 14: *Eleutherodactylus coqui*, 15: *Rhinoderma darwinii*, 16: *Litoria aurea*, 17: *Pseudacris maculata*, 18: *Hyla molleri*, 19: *Epidalea calamita*, 20: *Bufo spinosus*, 21: *Bufo bufo*, 22: *Rhinella schneideri*, 23: *Rhinella rubescens*, 24: *Anaxyrus fowleri*, 25: *Anaxyrus boreas*, 26: *Ambystoma maculatum*, 27: *Salamandra salamandra*, 28: *Pleurodeles waltl*, 29: *Paramesotriton hongkongensis*, 30: *Triturus marmoratus*, 31: *Triturus carnifex*, 32: *Triturus cristatus*, 33: *Notophthalmus viridescens*, 34: *Gyrinophilus porphyriticus*, 35: *Plethodon jordani*, 36: *Plethodon cinereus*. (C) Variation of ratio [(male aging rate)/(female aging rate)] in XY and ZW systems. We also provide the posterior probability distribution (curve filled in gray) of the effect size for the sex chromosome system on the ratio [(male aging rate)/(female aging rate)] obtained from Bayesian univariate mixed models (mean estimates and 95% CI are shown in full and dashed lines, respectively). (D) Posterior probability distribution of aging rate in heterogametic (XY) and homogametic (ZZ) males from the bivariate mixed model; the posterior distribution probability on the left shows the effect size for sex chromosome system across males. (E) Posterior probability distribution of aging rate in heterogametic (ZW) and homogametic (XX) females from the bivariate mixed model; the posterior distribution probability on the left shows the effect size for sex chromosome system across females.

Discussion

Our results showed that the genetic sex-determination system is associated with sex differences in aging rates, using amphibians as model species. In closely related taxa with XY and ZW systems, the heterogametic sex tended to show consistently higher aging rates. By contrast, we did not detect any effect on the sex chromosome system on sex-specific variation in lifespan, a mortality metric moderately correlated with aging rates in our samples of 36 amphibian species.

STRENGTHS AND LIMITS OF THE STUDY

We assembled a large longitudinal dataset for 36 species of anurans and urodeles, combining capture–recapture histories of >56,000 individuals marked, making this demographic dataset the broadest ever compiled so far for amphibians. Yet, despite this large sample size, our study displays several intrinsic limits. First, XY systems are far more common than ZW systems in extant amphibians (Perkins et al. 2019), which leads to strong imbalance of observations in XY (27 species) and ZW systems (9 species) in our analyses. However, the use of a Bayesian approach to test for associations between sex heterogamy and sex-specific senescence rate and lifespan have allowed us to take into account this disequilibrium among sex determination systems and small sample size while controlling for phylogenetic relationships among species.

Furthermore, the number of amphibian species for which the sex determination system has been described is still very limited (less than 400 species over the 8000 known species; Perkins et al. 2019; Ma and Veltsos 2021). This lack of knowledge led us to perform data imputation for 21 of the 36 studied species using Bayesian stochastic mapping. Imputation seems robust in families and genera that are the most represented in our study, whereas it seems to be less precise (posterior probability ≤ 0.7) in five species with XY systems (i.e., *Notophthalmus viridescens*, *Pelobates cultripes*, *Pelobates fuscus*, *Plethodon jordani*, and *Plethodon cinereus*; Supporting Information Table S2). Additional analyses (Supporting Information S4) nevertheless showed that association between senescence rates and sex heterogamy remained statistically significant even when these five species were removed from the analysis, or when the probability of sex chromosome assignment was included as explanatory response.

Finally, recent findings suggest that sex reversal could be widespread among amphibian populations in the wild (Lambert et al. 2019; Nemesházi et al. 2020). Thermal and chemical disturbances during embryonic or larval development can cause sex reversal, meaning that genetically female individuals become phenotypic males and vice versa (Flament 2016; Ruiz-García et al. 2021). The two studies that have evaluated this issue in nature show that the frequency of sex reversal may vary from 0.02 to 0.20 among anuran populations (Lambert et al. 2019; Nemesházi et al. 2020). Therefore, we assume that, in the worst-case scenario, 80% of phenotypic sexes inferred from secondary sexual characters (e.g., nuptial pads in anuran males, crests, and tail filament in urodele males) correspond to genotypic sexes in the populations included in our study. These discrepancies between phenotypic and genotypic sexes could not have produced the patterns we highlighted in our analyses, although errors of genotypic sex identification (up to 20% of misidentification according to Nemesházi et al. 2020) would introduce noise into the dataset so weakening the correlation between aging rate and sex determination system. Overall, although our study suffers from some deficiencies, it is based on the best demographic data currently available and provides the first preliminary evidence of a link between sex chromosome and aging rates in the animal kingdom.

SEX HETEROGAMY AND SENESCENCE RATE

Our findings indicate that sex differences in aging rates depend on sex chromosome systems. Although sex-specific variation within XY and ZW systems were small, possibly due to a lack of statistical power, aging rates tended to be higher in the heterogametic sex from both XY and ZW systems. This consistent trend across sex determination systems led to a small but detectable effect of sex chromosome system on sex differences in aging rates. The slight increase in aging rates shown by the heterogametic sex could be the result of two nonmutually exclusive molecular mechanisms, namely the "unguarded-X/Z" hypothesis (Trivers 1985; Marais et al. 2018) and the "toxic Y/W" hypothesis (Marais et al. 2018, 2020; Brown et al. 2020). The "unguarded X/Z" and "toxic Y/W" effects are expected to operate more strongly in sex chromosome systems with large nonrecombining regions and old and degraded Y/W chromosomes (Marais et al. 2018). Endothermic vertebrates typically have heteromorphic sex chromosomes with almost full X/Z-hemizygosity and highly repeat-rich Y(W) chromosomes in the heterogametic sex, with presumably ample opportunity for "unguarded X/Z" and "toxic Y/W" effects (Bellot et al. 2017; Peona et al. 2020). In contrast, the sex chromosomes of many ectothermic vertebrates including amphibians are often homomorphic or show subtle morphological differentiation when observed in light microscopy (Schartl et al. 2016; Pennell et al. 2018). Although amphibian sex chromosomes are far less well characterized than mammalian or avian ones (Miura 2017), the frequent incidence of homomorphy and mild heteromorphy indicates that amphibian sex chromosomes exhibit weak differentiation. The recent sequencing of a giant salamander genome and its sex chromosomes has confirmed this view (Keinath et al. 2018). It is therefore remarkable that we observed an association between sex chromosome systems and aging rate in amphibians.

If sex chromosomes are only weakly differentiated, how would the "unguarded X/Z" or "toxic Y/W effects" operate?

Achiasmy or heterochiasmy may be unusually frequent in amphibians (Sardell and Kirkpatrick 2020). If recombination is completely suppressed in males, or clustered at telomeric regions, a Y chromosome will undergo complete or nearly complete suppression of recombination from the beginning of its evolution, and start to degenerate over most or all of its length. The effect can be so strong that it might drive sex chromosome turnover like in ranid frogs (Jeffries et al. 2018). Accordingly, changing sex chromosomes may offer an evolutionary mechanism to purge the genome from deleterious mutations that accumulate on Y chromosomes. Another way of reducing the mutational load on Y chromosomes is through X-Y recombination (Dufresnes et al. 2015; Rodrigues et al. 2018). In *Hyla* treefrogs and *Rana temporaria*, X-Y recombination is suppressed in XY males, but is present in rare, sex-reversed XY females; so that, despite their rarity, occasional X-Y recombination events are sufficient to prevent degeneration of the Y chromosome (Stöck et al. 2011, 2013; Dufresnes et al. 2015; Rodrigues et al. 2018). We therefore propose that the opportunity for "unguarded X/Z" and "toxic Y/W" effects to operate might be as strong in amphibians as in mammals and birds, not because of strong heteromorphy but because of achiasmy and strong heterochiasmy. The effects should, however, be weaker in species that have recently turned over sex chromosomes or X-Y recombination.

SEX HETEROGAMY AND LIFESPAN

Lifespan was weakly negatively correlated to aging rate in our study ($R^2 < 0.27$), which is in line with the pattern observed in mammals where aging rate consistently explained less than half the variance in lifespan (Péron et al. 2019). Furthermore, we did not detect any effect on the sex chromosome system on sex-specific variation in lifespan, a mortality metric phylogenetically more conserved (H^2: 0.56 and 0.54 in males and females, respectively) than the aging rate (H^2: 0.22 and 0.24 in males and females, respectively) in our samples of 36 amphibian species. These results contrast with the findings of Xirocostas et al. (2020) that the heterogametic sex generally suffers from a shorter lifespan. This discrepancy could result from two main methodological causes: Xirocostas et al. (2020) only used lifespan metrics (i.e., mean, median, and maximal lifespan) that included the immature stage, whereas our lifespan metric was restricted to reproductive adults. Second, the phylogenetic coverage of the two studies broadly differs because Xirocostas et al. (2020) mainly focused on vertebrate lifespan data (72% of the species considered) strongly biased toward endotherms (92% of the vertebrate species) where amphibians were largely underrepresented (only seven species included, vs. 36 in our study). Overall, we suggest that current evidence does not allow us to detect the influence of sex chromosome systems on sex-related differences in lifespan in vertebrates. This question should be investigated more thoroughly in taxonomic groups that include variation in the heterogametic sex such as amphibians.

Conclusions

We compiled a large dataset of long-term capture–recapture data for amphibian clades containing both XY and ZW systems. Although our findings remain preliminary due to technical and data limitations, they strongly suggest that the evolution of sex-specific aging rates, but not adult lifespan, is partially driven by sex chromosomes. Despite having largely homomorphic, recently evolved and poorly differentiated sex chromosomes, amphibians may nonetheless be affected by the "unguarded X/Z" and/or "toxic Y/W" effects. Which mechanism operates under what conditions is yet to be clarified. In particular, it is currently unclear whether the "unguarded X/Z" may operate in recently evolved systems, where there has been little or no gene loss on the Y chromosome (and X-/Z-hemizygosity is absent). However, the accumulation of transposable elements can be fast (e.g., Schartl et al. 2016), which suggests that the "toxic Y/W" effect might impact incipient sex chromosome systems more strongly. Future theoretical and empirical work should address this issue preferably with the focus on taxa with atypical sex determination systems (such as X0, Z0, X1X2Y, XY1Y2, ZW1W2, and WXZ in fishes and reptiles; see Cioffi et al. 2017; Alam et al. 2018).

AUTHOR CONTRIBUTIONS

H.C. wrote the paper. H.C. and J.P.L. made the statistical analyses. H.C. initiated the project, and conceptualized and coordinated the work. J.F.L., J.M.G., G.M., C.V., and D.A.W.M. contributed to the writing of the article. All authors except H.C. J.F.L., J.M.G., G.M., and C.V. provided mark-recapture data. All authors read and edited the final manuscript version.

ACKNOWLEDGMENTS

We thank researchers, technicians, and students as all everyone who contributed to collect the capture–recapture data used our study. We also thank Thomas Flatt (Associate Editor) and the three anonymous reviewers for their useful and constructive comments. HC was supported as a postdoctoral researcher by the Vanier-Banting postdoctoral fellowship program and the Swiss National Science Foundation (SNF grant number 31003A_182265). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. This manuscript is contribution #782 of the USGS Amphibian Research and Monitoring Initiative.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

The data reported in this article are available in Supporting Information S1.

LITERATURE CITED

Alam, S. M. I., S. D. Sarre, D. Gleeson, A. Georges, and T. Ezaz. 2018. Did lizards follow unique pathways in sex chromosome evolution? Genes 9:239.

Austad, S. N., and K. E. Fischer. 2016. Sex differences in lifespan. Cell Metab. 23:1022–1033.

Bachtrog, D. 2013. Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. Nat. Rev. Genet. 14:113–124.

Bachtrog, D., J. E. Mank, C. L. Peichel, M. Kirkpatrick, S. P. Otto, T. L. Ashman, M. W. Hahn, J. Kitano, I. Mayrose, R. Ming, et al. 2014. Sex determination: why so many ways of doing it? PLoS Biol. 12:e1001899.

Bellott, D. W., H. Skaletsky, T. J. Cho, L. Brown, D. Locke, N. Chen, S. Galkina, T. Pyntikova, N. Koutseva, T. Graves, et al. 2017. Avian W and mammalian Y chromosomes convergently retained dosage-sensitive regulators. Nat. Genet. 49:387.

Brown, E. J., A. H. Nguyen, and D. Bachtrog. 2020. The Y chromosome may contribute to sex-specific aging in *Drosophila*. Nat. Ecol. Evol. 4:853–862.

Cioffi, M. D. B., C. F. Yano, A. Sember, and L. A. C. Bertollo. 2017. Chromosomal evolution in lower vertebrates: sex chromosomes in neotropical fishes. Genes 8:258.

Clutton-Brock, T. H., and K. Isvaran. 2007. Sex differences in ageing in natural populations of vertebrates. Proc. R. Soc. B Biol. Sci. 274:3097–3104.

Colchero, F., and J. S. Clark. 2012. Bayesian inference on age-specific survival for censored and truncated data. J. Anim. Ecol. 81:139–149.

Colchero, F., O. R. Jones, and M. Rebke. 2012. BaSTA: an R package for Bayesian estimation of age-specific survival from incomplete mark–recapture/recovery data with covariates. Methods Ecol. Evol. 3:466–470.

Dufresnes, C., A. Borzée, A. Horn, M. Stöck, M. Ostini, R. Sermier, J. Wassef, S. N. Litvinchuck, T. A. Kosch, B. Waldman, et al. 2015. Sex-chromosome homomorphy in Palearctic tree frogs results from both turnovers and X–Y recombination. Mol. Biol. Evol. 32:2328–2337.

Dufresnes, C., S. N. Litvinchuk, B. Rozenblut-Kościsty, N. Rodrigues, N. Perrin, P.-A. Crochet, and D. L. Jeffries. 2020. Hybridization and introgression between toads with different sex chromosome systems. Evol. Lett. 4:444–456.

Flament, S. 2016. Sex reversal in amphibians. Sex. Dev. 10:267–278.

Gelman, A., and D. Rubin. 1992. Inference from iterative simulation using multiple sequences. Statist. Sci. 7:457–511.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J. Statist. Softw 33:1–22.

Hadfield, J. D., and S. Nakagawa. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. J. Evol. Biol. 23:494–508.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. J. Theor. Biol. 12:12–45.

Hillis, D. M., and D. M. Green. 1990. Evolutionary changes of heterogametic sex and sex determination systems in the phylogenetic history of amphibians. J. Evol. Biol. 3:49–64.

Jeffries, D. L., G. Lavanchy, R. Sermier, M. J. Sredl, I. Miura, A. Borzée, L. N. Barrow, D. Canestrelli, P.-A. Crochet, C. Dufresnes, et al. 2018. A rapid rate of sex-chromosome turnover and non-random transitions in true frogs. Nat. Commun. 9:1–11.

Jetz, W., and R. A. Pyron. 2018. The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. Nat. Ecol. Evol. 2:850–858.

Keinath, M. C., N. Timoshevskaya, V. A. Timoshevskiy, S. R. Voss, and J. J. Smith. 2018. Miniscule differences between sex chromosomes in the giant genome of a salamander. Sci. Rep. 8:1–14.

Kowald, A. 2002. Lifespan does not measure ageing. Biogerontology 3:187–190.

Lambert, M. R., T. Tran, A. Kilian, T. Ezaz, and D. K. Skelly. 2019. Molecular evidence for sex reversal in wild populations of green frogs (*Rana clamitans*). PeerJ 7:e6449.

Lemaître, J. F., V. Ronget, M. Tidière, D. Allainé, V. Berger, A. Cohas, F. Colchero, D. A. Conde, M. Garratt, A. Liker, et al. 2020a. Sex differences in adult lifespan and aging rates of mortality across wild mammals. Proc. Natl. Acad. Sci. 117:8546–8553.

Lemaître, J. F., M. Garratt, and J. M. Gaillard. 2020b. Going beyond lifespan in comparative biology of aging. Adv. Geriatr. Med. Res. 2: e200011.

Liker, A., and T. Székely. 2005. Mortality costs of sexual selection and parental care in natural populations of birds. Evolution 59:890–897.

Ma, W. J., and P. Veltsos. 2021. The diversity and evolution of sex chromosomes in frogs. Genes 12:483.

Maklakov, A. A., and V. Lummaa. 2013. Evolution of sex differences in lifespan and aging: causes and constraints. BioEssays 35:717–724.

Marais, G. A., J. M. Gaillard, C. Vieira, I. Plotton, D. Sanlaville, F. Gueyffier, and J. F. Lemaitre. 2018. Sex gap in aging and longevity: can sex chromosomes play a role? Biol. Sex Differ. 9:33.

Marais, G., J. F. Lemaitre, and C. Vieira. 2020. Y chromosome makes fruit flies die younger. Peer Commun. Evol. Biol. 1:100105.

Miura, I. 2017. Sex determination and sex chromosomes in Amphibia. Sex. Dev. 11:298–306.

Nemesházi, E., Z. Gál, N. Ujhegyi, V. Verebélyi, Z. Mikó, B. Üveges, K. K. Lefler, D. L. Jeffries, O. I. Hoffmann, and V. Bókony. 2020. Novel genetic sex markers reveal high frequency of sex reversal in wild populations of the agile frog (*Rana dalmatina*) associated with anthropogenic land use. Mol. Ecol. 29:3607–3621.

Pennell, M. W., J. E. Mank, and C. L. Peichel. 2018. Transitions in sex determination and sex chromosomes across vertebrate species. Mol. Ecol. 27:3950–03963.

Peona, V., O. M. Palacios-Gimenez, J. Blommaert, J. Liu, T. Haryoko, K. A. Jønsson, M. Irestedt, Q. Zhou, P. Jern, and A. Suh. 2020. The avian W chromosome is a refugium for endogenous retroviruses with likely effects on female-biased mutational load and genetic incompatibilities. bioRxiv. https://doi.org/10.1101/2020.07.31.230854

Perkins, R. D., J. R. Gamboa, M. M. Jonika, J. Lo, A. Shum, R. H. Adams, and H. Blackmon. 2019. A database of amphibian karyotypes. Chromosome Res. 27:313–319.

Péron, G., J. F. Lemaître, V. Ronget, M. Tidière, and J. M. Gaillard. 2019. Variation in actuarial senescence does not reflect life span variation across mammals. PLoS Biol. 17:e3000432.

Pipoly, I., V. Bókony, M. Kirkpatrick, P. F. Donald, T. Székely, and A. Liker. 2015. The genetic sex-determination system predicts adult sex ratios in tetrapods. Nature 527:91–94.

Promislow, D. E., R. Montgomerie, and T. E. Martin. 1992. Mortality costs of sexual dimorphism in birds. Proc. R. Soc. Lond. B Biol. Sci. 250:143–150.

Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol. Evol. 3:217–223.

Rodrigues, N., T. Studer, C. Dufresnes, and N. Perrin. 2018. Sex-chromosome recombination in common frogs brings water to the fountain-of-youth. Mol. Biol. Evol. 35:942–948.

Ronget, V., and J. M. Gaillard. 2020. Assessing ageing patterns for comparative analyses of mortality curves: going beyond the use of maximum longevity. Funct. Ecol. 34:65–75.

Ruiz-García, A., Á. S. Roco, and M. Bullejos. 2021. Sex differentiation in amphibians: effect of temperature and its influence on sex reversal. Sex. Dev. 15:1–11.

Sardell, J. M., and M. Kirkpatrick. 2020. Sex differences in the recombination landscape. Am. Nat. 195:361–379.

Schartl, M., M. Schmid, and I. Nanda. 2016. Dynamics of vertebrate sex chromosome evolution: from equal size to giants and dwarfs. Chromosoma 125:553–571.

Siler, W. 1979. A competing-risk model for animal mortality. Ecology 60:750–757.

Stöck, M., A. Horn, C. Grossen, D. Lindtke, R. Sermier, C. Betto-Colliard, C. Dufresnes, E. Bonjour, Z. Dumas, E. Luquet, et al. 2011. Ever-young sex chromosomes in European tree frogs. PLoS Biol. 9: e1001062.

Stöck, M., R. Savary, A. Zaborowska, G. Górecki, A. Brelsford, B. Rozenblut-Kościsty, M. Ogielska, and N. Perrin. 2013. Maintenance of ancestral sex chromosomes in Palearctic tree frogs: direct evidence from *Hyla orientalis*. Sex. Dev. 7:261–266.

Suchard, M. A., P. Lemey, G. Baele, D. L. Ayres, A. J. Drummond, and A. Rambaut. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 4:vey016.

Tidière, M., J. M. Gaillard, D. W. Müller, L. B. Lackey, O. Gimenez, M. Clauss, and J. F. Lemaître. 2015. Does sexual selection shape sex differences in longevity and senescence patterns across vertebrates? A review and new insights from captive ruminants. Evolution 69: 3123–3140.

Tree of Sex Consortium. 2014. Tree of sex: a database of sexual systems. Sci. Data, 1:140015.

Trivers, R. 1985. Social evolution. Benjamin/Cummings, Menlo Park, CA.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

Xirocostas, Z. A., S. E. Everingham, and A. T. Moles. 2020. The sex with the reduced sex chromosome dies earlier: a comparison across the tree of life. Biol. Lett. 16:20190867.

Associate Editor: T. Flatt
Handling Editor: A.G McAdam

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Summary of the capture-recapture data from the 36 amphibian species considered in our study.
Table S2. List of the species used for the Bayesian stochastic mapping.
Figure S1. Relationships between lifespan (median and 80%) and aging rate in females (A) and males (B).
Figure S2. Comparing lifespan distributions (i.e., median lifespan and lifespan 80%) according to sex and sex chromosome system.
Figure S3. Log-scaled ratio of aging rate between sexes is modeled as function of the probability of assignment to XY system.
Figure S4. Aging patterns and Siler model parameters in *Anaxyrus boreas* (A), *Anaxyrus fowleri* (B), *Bombina pachypus* (C), and *Bombina variegata* (D)
Figure S5. Aging patterns and Siler model parameters in *Bufo bufo* (A), *Bufo spinosus* (B), *Epidalea calamita* (C), and *Eleutherodactylus coqui* (D).
Figure S6. Aging patterns and Siler model parameters in *Gyrinophilus porphyriticus* (A), *Hyla molleri* (B), *Litoria aurea* (C), and *Rana areolata* (D).
Figure S7. Aging patterns and Siler model parameters in *Pelobates fuscus* (A), *Pelobates cultripes* (B), *Notophthalmus viridescens* (C), and *Pelophylax esculentus* (D).
Figure S8. Aging patterns and Siler model parameters in *Plethodon jordani* (A), *Plethodon cinereus* (B), *Pelophylax perezi* (C), and *Pleurodeles waltl* (D).
Figure S9. Aging patterns and Siler model parameters in *Rana cascadae* (A), *Rana luteiventris* (B), *Pseudacris maculata* (C), and *Rana muscosa* (D).
Figure S10. Aging patterns and Siler model parameters in *Rana sierrae* (A), *Rana temporaria* (B), *Rhinoderma darwinii* (C), and *Rhinella rubescens* (D).
Figure S11. Aging patterns and Siler model parameters in *Salamandra salamandra* (A), *Triturus carnifex* (B), *Triturus cristatus* (C), and *Triturus marmoratus* (D).