Title: Diverse aging rates in ectothermic tetrapods provide insights for the evolution of aging and longevity

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Abstract:

Comparative studies of mortality in the wild are necessary to understand the evolution of aging, yet ectothermic tetrapods are under-represented in this comparative landscape despite their suitability for testing evolutionary hypotheses. We provide the first comprehensive study of aging rates and longevity across tetrapod ectotherms in the wild, utilizing data from 107 populations across   77 species   of reptiles and amphibians. We tested hypotheses of how thermoregulatory mode, environmental temperature, protective phenotypes, and pace of life contribute to aging. Controlling for phylogeny and body size, ectotherms displayed a higher diversity of aging rates than endotherms, and included many groups with negligible aging. Protective phenotypes and life-history tactics further explained macroevolutionary patterns of aging. By including ectothermic tetrapods, our comparative analyses enhance our understanding of aging evolution.

One-Sentence Summary: Ectothermic tetrapods included many species with negligible aging in the wild; protective phenotypes (e.g., armor and venom), temperature, and life-history tactics co-varied   with macroevolutionary patterns of aging in ectotherms.

Main Text: Comparative studies of aging rates of animals in the wild are key   for assessing the potential limits of longevity   and understanding   ecological and evolutionary factors shaping variation in aging strategies (*1*, *2*). Demographic indicators of aging have generally focused on adult longevity   and changing   mortality rates   with advancing adult age. Past comparative studies have provided important insights regarding the evolution of demographic aging in the endothermic tetrapod groups of birds and mammals (e.g., *2–6*). However, ectothermic tetrapods (i.e.,   reptiles and amphibians) hold most of the records for animal longevity, and comprise   26 of the 30 known records for vertebrate species that can live over

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100 years (*7*, *8*) (examples include Galapagos tortoises, eastern box turtles, European pond turtles, and proteus salamanders). Additionally, some ectothermic tetrapods may exhibit low or even negligible mortality and reproductive aging (*1*, *9–14*). U nderstanding whether and how natural selection has shaped mortality trajectories and longevity requires testing if these species-specific results are anomalies that evolved in specific lineages, or if they are common and repeated evolutionary outcomes. The lack of comparative analyses of aging in ectothermic tetrapods is a major gap in our knowledge, but it is not surprising; ectotherms are often secretive and/or seasonally active, and many species have long generation times and sparse population densities. Long-term data collection of ectotherms in the wild is thus especially challenging. A comprehensive analysis of aging across ectothermic tetrapods requires decades of field-based research, international collaborations, and powerful quantitative tools. Integrating these efforts allows for using ectotherms to test key evolutionary hypotheses of aging (*15*), and for a phylogenetic understanding of the evolution of aging in tetrapods.

The evolutionary genetics of aging hinge on age-specific mutation-selection balance trajectories where mutations have age-specific effects that are strictly deleterious in later adult stages or ages, or beneficial earlier (i.e., antagonistically pleiotropic, *16*). Hypotheses for how natural selection and the environment interact to shape this balance were first formulated by Medawar (*17*), and further developed by Hamilton (*18*) and others (*19–21*). In ectotherms, body temperature varies with the environment and, because metabolism responds to temperature, ectothermic metabolism and cellular processes can downregulate in cold temperatures, allowing for extended periods of brumation and aestivation. In addition, controlling for body size, ectotherms have lower resting metabolic rates than endotherms (23). Accordingly, the Thermoregulatory Mode Hypothesis predicts that ectothermic lineages have evolved lower aging

rates and greater longevity   than their similarly sized endothermic counterparts (*22*).

Layered on top of metabolic mode, temperature itself is expected to be a strong driver of

mortality, impacting both the evolution and the plasticity of aging (*24*, but see *25*). In

endotherms, species with lower body temperatures live longer and age slower than those with

higher body temperatures (*22*, *26*), so ectotherms in cooler climates may also exhibit longer

lifespans and slower aging compared to those in warmer climates (Temperature Hypothesis,

hereafter).

Phenotypes that alter age-specific mutation-selection trajectories would be expected to

result in the evolution of altered rates of aging (*18*), provided genetic variation exists (*21*, *27*).

For example, species with phenotypes that reduce mortality risk are expected to have lower rates

of aging than those without (*15*) (the so-called Protective Phenotypes Hypothesis). Previous

work shows that ectotherm groups with chemical protection mechanisms   can live longer than

those that do not, though how this trait affects the rate of aging remains unknown (*28*, *29*).

Tetrapod ectotherms are well-suited for enabling direct comparisons of the rates of aging among

species with and   without phenotypes that have such physical or chemical protections. Within

reptiles, diverse morphological traits may confer protection from predation, including turtle

shells, crocodilian armor, and snake venom (even if such traits are exaptations). Similarly, in

amphibians, many species produce toxic or unpalatable skin secretions (*30*). Despite this, the

Protective Phenotypes Hypothesis has not been tested broadly within a phylogenetic framework

(but see *28*).

Aging and longevity may co-evolve either through direct or indirect selection on life-

history traits that are genetically correlated. Under antagonistic pleiotropy, genes that confer

greater fitness in early life relative to late life will increase in frequency in populations that are

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skewed toward younger age classes (*18*). Because many ectothermic tetrapods have indeterminate growth and fecundity (*31*, *32*), life-history theory predicts that such species should have stronger selection against deleterious late-age mutations relative to species with determinate growth and fecundity. Indeed, any species in which   older age classes contribute relatively more to population growth (e.g., fecundity, behavior)   relative to other species should have concomitant slower aging. Thus, the aging   rate may evolve from genetic co   variation among life-history traits, such as annual fecundity, age of first reproduction, and longevity (i.e., traits that define the trajectory of individuals throughout their life). This results in a slow-fast continuum of life histories (*33–36*) that should match slow vs. fast aging rates (the Slow-Fast Continuum Hypothesis). For example, fast aging   expected to be correlated with a short reproductive lifespan   should evolve in a correlated manner with swift pace of life, and vice versa (*33*, *37*). Therefore, existence of a strong positive co   variation among biological times (e.g., *38*) predicts that the aging rate should co   vary with age of first reproduction (negatively) and with annual fecundity (positively) such that species that mature relatively early or that allocate relatively more energy to reproduction   display faster aging and shorter longevities (*35*, *39*).

We apply comparative phylogenetic methods to tetrapod data to analyze ectotherm aging and longevity in the wild, to compare to endotherms, and to address   the following four hypotheses for the evolution of aging in ectotherms: i) Thermoregulatory Mode, ii) Temperature, iii) Protective Phenotypes, and iv) Slow-Fast Continuum. We analyze long-term   capture-recapture data   collected in the wild from 107 populations of 77 species, with study length averaging 17 years (ranging from 4 to 60 years), to assess macroevolutionary patterns of mortality, aging and longevity in free-living amphibians and non-avian reptiles. We present the

first comprehensive comparative analysis of patterns of aging across these ectotherms, and analyze both the rate of aging (computed as the slope of the relative rate of increasing age-specific mortality derived from the Gompertz model, β_1) and longevity (computed as the number of years after the age of first reproduction at which 95% of individuals in a given adult cohort have died). Specifically, we test: i) whether ectotherms consistently age slower and live longer than endotherms; ii) whether mean, minimum, or maximum environmental temperature experienced by a population co-varies with the rate of aging and longevity; iii) whether species with protective phenotypes (either physical or chemical) age slower and live longer than those without physical or chemical protections; and iv) whether the rate of aging and longevity strongly co-vary with other biological traits, such as age at first reproduction and annual fecundity.

Aging in ectothermic tetrapods

All major ectothermic orders within 77 reptile and amphibian species examined had at least one species with negligible aging ($\beta_1 \sim 0$; Fig 1, Data S1). Notably, turtles had slow rates of aging (mean $\beta_1 \pm$ SE= 0.04 $\pm$ 0.01), with a small range relative to the number of species represented (-0.013 – 0.225 for 14 species; Fig 2, Table S1). Crocodilians, tuatara, and salamanders were similarly slow in aging (crocodilians: mean $\beta_1 = 0.14 \pm 0.06$, tuatara: 0.005, and salamanders: 0.18 ± 0.05) in comparison to squamates (mean $\beta_1 = 0.55 \pm 0.14$) and frogs (mean $\beta_1 \pm$ SE $= 0.41 \pm 0.06$; Fig 2, Table S1-S2, Data S1). Turtles and tuatara had greater longevity (95% of adult lifespan) than most other ectothermic tetrapods, with mean longevities of 39 ($\pm$ SE 6) and 137 years, respectively, compared to crocodilians (21 years $\pm$ 5), squamates (12 years $\pm$ 2), frogs (8 years $\pm$ 0.6), and salamanders (10 years $\pm$ 1; Table S1-S2, Data S1).

Thermoregulatory Mode Hypothesis

Controlling for phylogeny and body size, across tetrapods, aging rate and longevity did not differ between ectotherms and endotherms (Table 1, Fig 3, see Fig S1 for raw values by class). Ectotherms ranged above and below the known aging rates for endotherms ($C_v = 1.40$ for ectotherms and 1.15 for endotherms), and had the greatest longevities ($C_v = 0.37$ for ectotherms and 0.32 for endotherms; Fig S1). Ectotherm variance in aging rate was significantly different from the endotherm variance ($F_{106/118} = 5.49$, p =< 0.001), although the variances in longevities were not different ($F_{106/118} = 1.31$, p = 0.16). There was a negative relationship between aging rate and longevity for both groups, with faster aging rates corresponding to shorter longevity, but the slope of the relationship was lower in ectotherms than in endotherms (Table 1, Fig 3C). The negative association between rate of aging and longevity varied considerably among mammals, birds, reptiles, and amphibians, when considered by taxonomic class (Fig S2; Table S3).

Temperature Hypothesis

Within ectotherms, the rate of aging increased with mean temperature in reptiles, but decreased with mean temperature in amphibians (Table 2, Fig S3). Models using minimum and maximum temperatures instead of mean showed the same patterns (Table S4).

Protective Phenotypes Hypothesis

We considered three categories of protection: physical (armor and shells), chemical (venom and skin toxins), and neither physical nor chemical (Fig S4). Within ectothermic tetrapods, species with physical or chemical protection aged slower than species with neither physical nor chemical protection (mean $\beta_1 \pm$ SE: 0.05 ± 0.01, 0.29 ± 0.06, 0.47 ± 0.07, respectively). Species with physical protection lived longer than those with no protection and

those with chemical protection (mean years ± SD: 36 ± 5, 10 ± 3, 11 ± 1, respectively, Table S5, Data S1).

Slow-fast Continuum Hypothesis

We examined relationships between both the age of first reproduction and annual fecundity and rate of aging and longevity. The rate of aging was negatively associated with the log age of first reproduction, and positively associated with the log annual fecundity (Table 2). However, because class (i.e., reptiles or amphibians) was significant, we further investigated these trends per class. We found that the pattern of a decreasing rate of aging with increasing age of first reproduction was driven by reptiles, whereas the increasing rate of aging with increasing annual fecundity was driven by amphibians (Table S6, Fig 4A-4B). Longevity was positively associated with the age of first reproduction, with similar magnitude in both amphibians and reptiles (Table 2, Fig. 4C). Longevity was not related to annual fecundity (Table 2, Fig 4D).

Discussion

We found greater variation in aging rates and longevity in the wild across ectothermic tetrapods than in birds and mammals. Our comprehensive study also offers robust insight that the parameter space for aging rates and longevity is much larger than previosuly thought, and that turtles, crocodilians, and salamanders have remarkably low aging rates and extended longevities for their size. Most turtles have physical protection (bony shells, which are external rib cages), as well as a relatively slow pace of life, which both contribute to the negligible mortality and exceptional longevity. In addition, aging rates remained low overall, although for at least one s turtle (*Chrysemys picta*), age at maturation, longevity and aging rates varied among

populations (8, 11, 12, this study). Future work that focuses on turtles with soft shells (versus rigid, as in this study) may   help disentangle causes of slow turtle aging.

Our analyses provide clear evidence that ectotherms have a remarkable diversity of aging rates and longevity   . Within ectotherms, rate of aging ranged from -0.013 to 2.0, corresponding to a continuum from negligible senescence to   very fast aging. Ectotherm lifespan ranged from 1 year to 137 years. In comparison, the human   aging rate is about 0.1, with maximum longevity of 110 years (*2*). Within   Primates, aging rates range between   0.04 – 0.5 (longevity: 4 – 84 years), and the overall m   ammalian rate of aging ranges from 0 to 1.2, with a   single high value observed in eastern moles (*Scalopus aquaticus*) representing   an outlier (Fig S1). One notable group of vertebrates missing from our comparisons   are fishes, which themselves have highly variable aging rates and longevities and contain species of great interest to aging biology (e.g., rock fish, B   igmouth buffalo, and short-lived poeciliids) (1, *34–36*).

In addition to expanding the domain for aging research and gaining insights into ectotherm aging, we used   novel data to test four hypotheses on the evolution of aging in a comparative framework. Our test of the Thermoregulatory Mode Hypothesis revealed that across tetrapods, ectotherms did not have slower rates of aging (Adjusted $R^2 = 0.05$, $p = 0.26$) or longer lifespans (Adjusted $R^2 = 0.20$, $p = 0.64$) than similar-sized endotherms. However, thermoregulatory mode   appeared   to modulate the relationship between aging rate and longevity (when phylogenetically and body-mass controlled: Adjusted $R^2 = 0.38$, Fig 3C).

We found mixed support for the Temperature Hypothesis as it relates to rate of aging; mean temperature interacted with class such that the rate of aging increased with mean temperature in reptiles, but decreased with mean temperature in amphibians (Fig S3, Table 2).

Moreover, this   interaction corresponded to the same directionalities when we tested for a relationship with minimum or maximum temperature (Table S4). We found no associations between longevity and   mean, minimum, or maximum temperature. Because temperature is a proximate mediator of cellular and biochemical processes, it is also likely a driver of local adaptation among populations – and plasticity within individuals – for phenotypes related to aging and longevity (*24*, reviewed in *43*).

Temperature increases ectotherm metabolic rate and putatively hastens accumulation of molecular damages via multiple processes, such as free radical production, telomere attrition, secretion of cytokines from senescent cells, and DNA damage (*43*). For example, in garter snakes (Thamnophis spp.) and frogs, thermal differences among populations have been hypothesized to be an agent of selection for life-history divergence, including aging (*25*, *44*). Laboratory experiments that raise ectotherms under different thermal regimes can directly test for the proximate effect of temperature on aging (e.g., *45*), and are necessary to tease apart how temperature might influence the evolution of aging. Also, global warming may be a   driver of longevity/aging in ectotherm   s. Because it is an ongoing effect   (IPCC, 2021) and   can accelerate rates of senescence in these organisms (Stark et al., 2020),   management and conservation strategies may   be even more necessary to avoid species extinction.

Our analyses also provided clear evidence supporting the Protective Phenotypes Hypothesis within ectothermic tetrapods. Species with physically protective phenotypes, such as armor, spines, or shells, aged more slowly and lived much longer for their size than those without protective phenotypes (Table S5). Though species with chemical protection have greater longevity   than those without (*29*, *46*), this is the first time a metric of senescence - rate of aging - has been linked to these protective phenotypes. This result may explain uniquely slow

rates of aging in turtles, coupled with extended longevity . Salamanders also aged slowly

relative to other tetrapod ectotherms. We were unable to include behaviors, such as fossorial

lifestyles or seasonal activity, that may function as behavioral protections by reducing predation

risk, with a consequence of low mortality rates. Moreover, many salamanders have regenerative

capabilities that could contribute to slowing aging through greater damage repair efficiency (*47*,

48).

Lastly, we document that mortality aging is involved in shaping the slow-fast continuum

of life histories. Both rates of aging and longevity were associated with other biological traits

in reptiles and amphibians. Earlier age at first reproduction in reptiles was correlated with faster

aging rates (Table 2, Fig 4). A similar pattern has been documented in birds and mammals,

where an earlier age at first reproduction corresponded to an earlier age at the onset of

senescence (*49*, *50*). Amphibian species with larger annual fecundities, and therefore greater

annual reproductive allocation, had faster rates of aging, which has also been found in birds and

mammals, and supports Hamilton's original prediction (*18*). Earlier age of first reproduction was

also associated with shorter longevity in both amphibians and reptiles (Fig 4). Heralded as a key

component of the life-history portfolio (*51*, *52*), this positive relationship between age at first

reproduction and adult longevity is thus robust across tetrapod ectotherms as well. These results

are congruent with patterns detected in endothermic vertebrates (*3*) and fit into an existing

evolutionary framework of genetic correlations underlying relationships among life-history traits,

including aging and longevity. Further work on the quantitative genetic and genomic bases of

aging and longevity are necessary to test whether the phenotypic associations are underlain by

genetic correlations (25).

The evolution of aging rates and longevity have seemingly multiple determinants from the genetic architecture of life-history traits to morphological adaptations, yielding complex aging patterns across free-ranging tetrapods and, truly, the tree of life (*1*). Long-term studies of species from wild populations are necessary for understanding such complexity in the natural context in which aging evolved (*53*). O   ur comprehensive compilation of long-term field studies clarifies mechanisms underlying the evolution of aging rate in tetrapod vertebrates, highlighting links among protective phenotypes, life-history tactics, and aging variation in the wild.

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Data and materials availability: Data and code used for these analyses are available in Dryad (#TBD). Individual mark-recapture datasets must be obtained by contacting specific dataset owners (see Data S1).

Supplementary Materials

Materials and Methods

Supplementary Text

Figs. S1 to S7

Tables S1 to S7

References (55-76)

Data S1
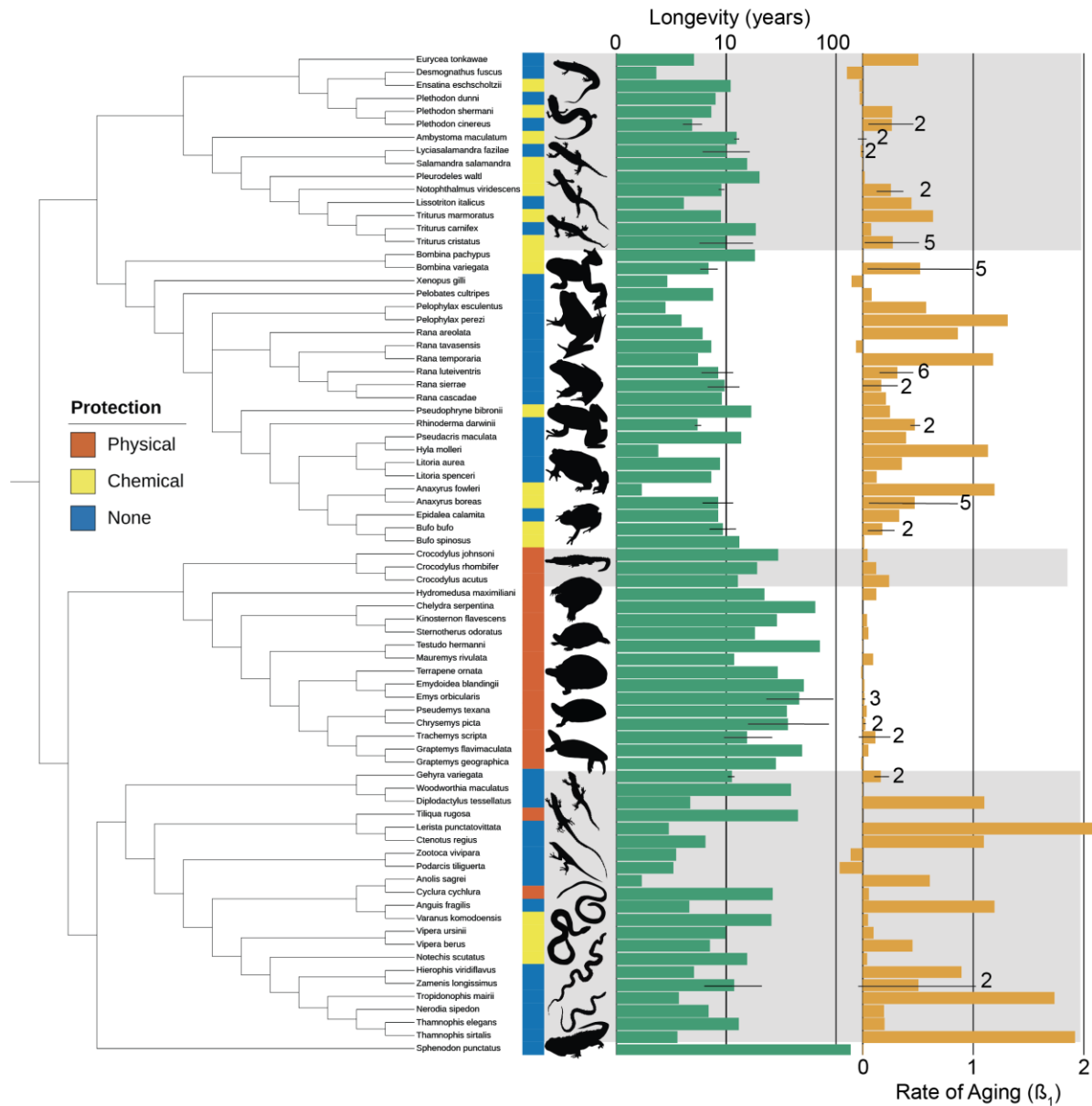
Fig. 1. Tetrapod ectotherms and their measures of aging. The rate of aging is the
Gompertz slope parameter indicating how mortality risk increases with age (in number of years
since first reproduction)   . Longevity is the number of years from the age of first
reproduction at which 95% of the individuals in a population have died. Error bars show +/-1 SD
for species for which multiple populations were analyzed. Shading represents taxonomic orders.

30

The number next to the bar represents the number of populations included in this study. Figure was made with iTOL (*54*) and silhouettes are available on phylopic.org.
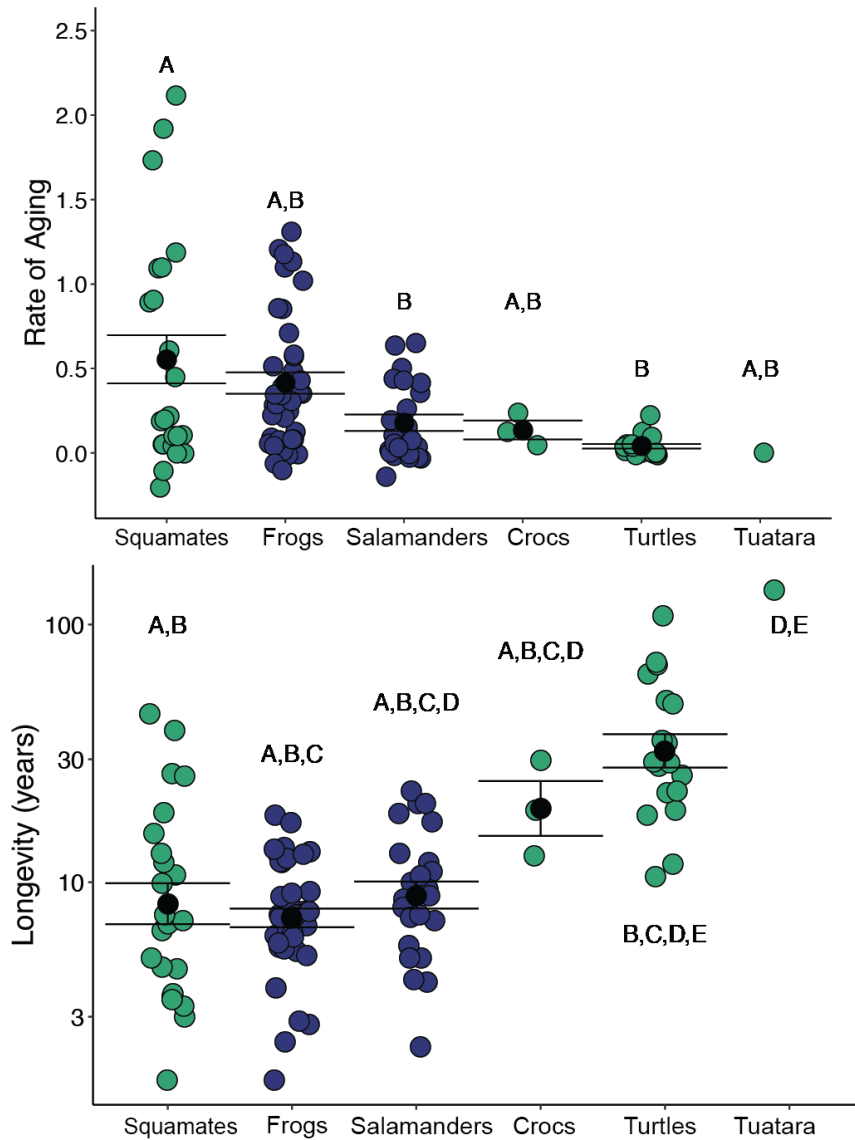
Fig. 2. Measures of rates of aging and longevity across ectotherms. Letters denote statistical significance across orders after correcting for body mass and phylogeny (Table S2). Bars show +/- 1 SE. Points are uncorrected values for visualization. The rate of aging here is the mortality slope derived from a Gompertz model. Longevity is the number of years from the age of first

reproduction at which 95% of the individuals in a population have died. Green denotes reptiles

and purple denotes amphibians.
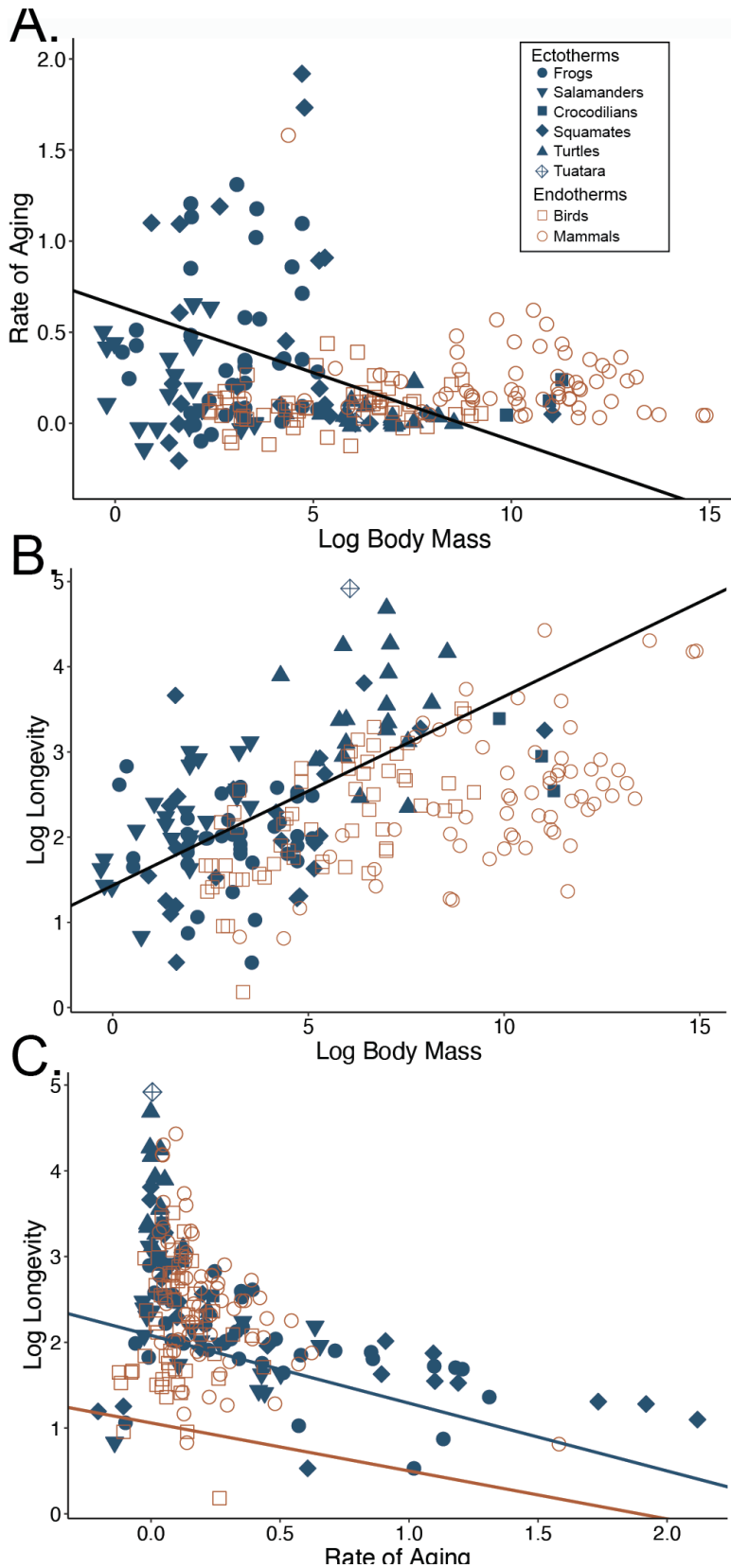
Fig. 3. Comparison of ectothermic versus endothermic tetrapods for (A) rates of aging, (B) longevity, and (C) the relationship between aging rate and longevity. Trend lines are derived from PGLS models. Orange denotes endotherms and blue denotes ectotherms. Black

lines in A and B show the overall effect (no difference between endotherms and ectotherms). See

Table 1 for P-values of these interactions.
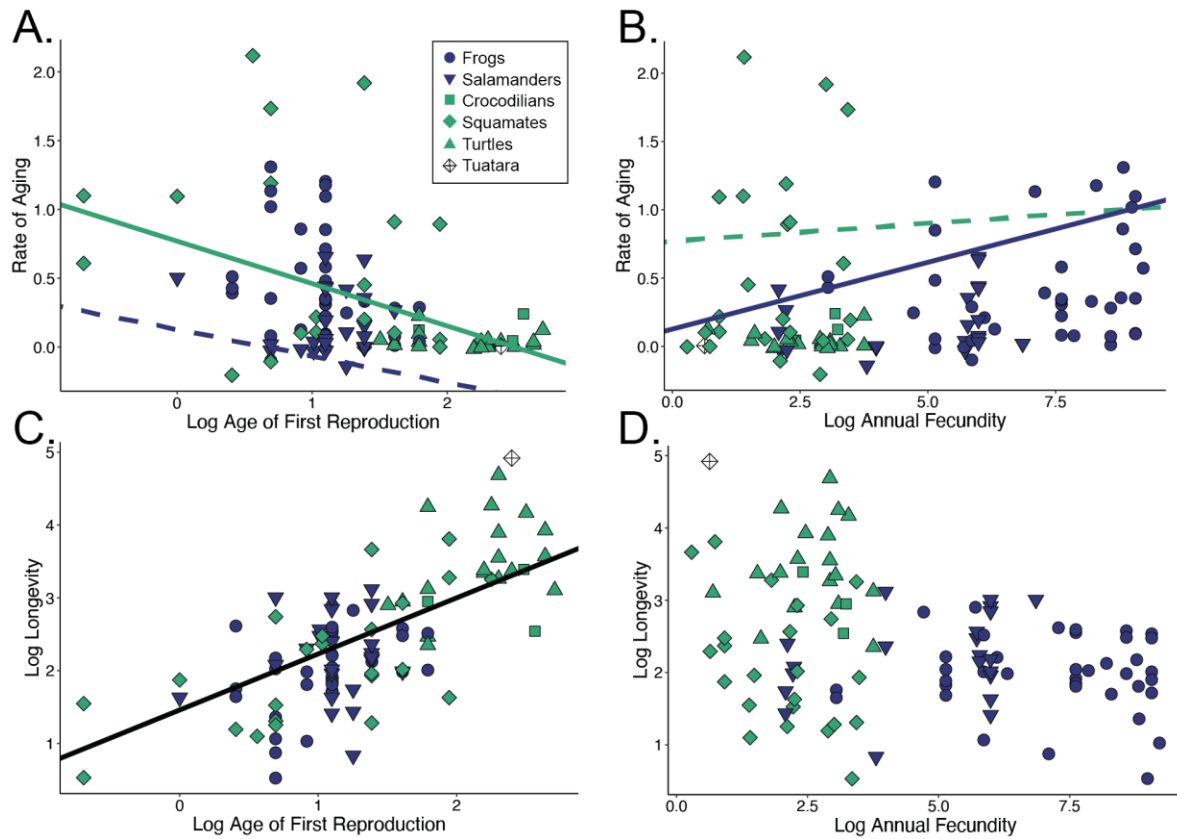
Fig 4. Slow-fast Continuum Hypothesis. Solid lines show statistically significant relationships between variables and are derived from PGLSs from Table 2. Dashed lines are included for visualizing the contrasting class. Green denotes reptiles and purple denotes amphibians. The black line in C denotes the overall effect (no difference between reptiles and amphibians). Age at first reproduction and annual fecundity themselves did not differ by class (when controlling for phylogeny and body mass; Table S7).

Table 1. Statistical output for PGLSs and phylogenetic ANCOVAs comparing ectotherms and endotherms for the Thermoregulatory Mode Hypothesis. Group is a factor with two levels: ectotherms vs. endotherms. Interaction (bold) terms denote group differences after correcting for body mass plotted in Fig 3. These were not significant for rate of aging or longevity (see Fig 3).

Model	Df	Sum Sq	Mean Sq	F value	Est	P-value
Ectotherms vs Endotherms						
Rate of aging (Adj R^2 =0.06)						
Group	1	0.01	0.01	0.001	-0.39	0.76
Log mass	1	129.00	129.00	14.46	-0.08	<0.001
Log mass × group	1	16.44	16.44	1.84	0.04	**0.18**
Residuals	222	1979.85	8.92			
Log longevity (Adj R^2 =0.20)						
Group	1	1.96	1.96	0.08	-0.30	0.89
Log mass	1	1479.83	1479.83	59.16	0.22	<0.001
Log mass × group	1	6.83	6.83	0.27	-0.03	**0.60**
Residuals	222	5620.40	25.32			
Log longevity (Adj R^2 =0.37)						
Rate of aging	1	1787.63	1787.63	90.24	-0.87	<0.001
Group	1	2.23	2.23	0.11	-0.63	0.74
Log mass	1	851.72	851.72	42.99	0.17	<0.001
Rate of aging × group	1	107.47	107.47	5.43	0.56	**0.02**
Residuals	221	4378.00	19.81			

Table 2. Statistical output for ectotherm PGLSs showing output of all predictor variables for the Temperature, Protective Phenotypes, and Slow-fast Continuum Hypotheses.

Protection is a factor with three levels: none, chemical, and physical. Class is a factor with two levels: reptile and amphibian. Bold P-values correspond to tests of the specific hypothesis in question.

PGLS Model	Df	Sum Sq	Mean Sq	F value	Est	P-value
Temperature Hypothesis						
Rate of aging (Adj R^2 = 0.06, λ = 0)						
Class	1	0.01	0.01	0.05	-0.28	0.18
Mean temp	1	0.003	0.003	0.002	-0.002	**0.08**
Class × mean temp	1	1.05	1.05	5.56	0.004	0.02
Log mass	1	1.01	1.01	5.39	-0.07	0.003
Residuals	102	18.54	0.20			
Log longevity (Adj R^2 = 0.15, λ = 0.67)						
Class	1	0.72	0.72	0.66	0.42	0.71
Mean temp	1	1.23	1.23	1.12	-0.001	**0.51**
Class × mean temp	1	0.18	0.18	0.17	-0.001	0.66
Log mass	1	22.34	22.34	20.41	0.18	<0.001
Residuals	102	109.44	1.07			
Protective Phenotypes Hypothesis						
Rate of aging (Adj R^2 = 0.11, λ = 0)						
Protection	2	2.79	1.40	7.85	------	**<0.001**
Log mass	1	0.13	0.13	0.75	0.02	0.39
Residuals	103	18.34	0.18			
Log longevity (Adj R^2 = 0.44, λ = 0)						
Protection	2	35.25	17.62	42.15	------	**<0.001**
Log mass	1	1.25	1.25	3.00	0.06	0.09
Residuals	103	43.06	0.42			

Slow-fast Continuum Hypothesis						
Rate of aging (Adj $R^2 = 0.18$, $\lfloor = 0$)						
Log age at repro	1	1.08	1.08	6.48	-0.26	**0.01**
Log annual fecundity	1	0.39	0.39	2.34	0.07	**0.04**
Class	1	0.28	0.28	1.71	0.46	0.02
Log mass	1	2.78	2.78	16.73	-0.03	0.41
Residuals	99	16.44	0.17			
Log longevity (Adj $R^2 = 0.52$, $\lfloor = 0$)						
Log age at repro	1	9.75	9.75	26.34	0.77	**<0.001**
Log annual fecundity	1	8.89	8.89	24.00	-0.06	**0.22**
Class	1	4.20	4.20	11.34	-0.06	0.85
Log mass	1	19.38	19.38	52.32	0.05	0.32
Residuals	99	36.66	0.37			

Science

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Supplementary Materials for

Diverse aging rates in ectotherms provide insights for the evolution of aging and longevity

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Materials and Methods
Supplementary Text
Figs. S1 to S7
Tables S1 to S6

Captions for Data S1

Other Supplementary Materials for this manuscript include the following:
Data S1
Materials and Methods
To test the Thermoregulatory Mode, Temperature, Protective Phenotypes, and Slow-Fast
Continuum Hypotheses, we used the rate of aging (Gompertz slope parameter) and longevity
(number of years after age of first reproduction at which 95% of the individuals in the adult
population have died) of ectothermic tetrapods from long-term capture-recapture datasets for
wild populations of amphibians and reptiles around the world, in addition to population-level
temperature data and species-level trait data. For the Thermoregulatory Mode Hypothesis, and to
compare endotherms and ectotherms in general, aging rates and longevities for mammals and
birds were obtained from published datasets. We then used a supertree and phylogenetic
generalized least squares regression (PGLS) models to test the above hypotheses of aging in a
phylogenetic framework.
Ectotherm datasets
        We gathered long-term amphibian and reptile datasets from around the world (Fig S5;
Data S1) to compare aging rates and longevities in ectotherms. These datasets represent 77
species from 107 populations on six   continents (all continents on which tetrapod ectotherms
naturally occur). Encounter histories were assembled from the capture data and when animals
were of known age, the year of birth was included in the dataset. In some cases, data were
collected from multiple adjacent populations. We combined data when similar capture effort
occurred at all locations over the same time span. If not, we only included data from the
population with highest capture rates. For all datasets, we analyzed adult aging rate beginning at
age of maturation, and adult longevity (i.e., 95% longevity minus the age of first reproduction).
Finally, while some datasets included accurate information about sex for all individuals, the
majority did not have this information for all individuals because of challenges in determining
sex from external characteristics of juveniles and adults, in some cases. Therefore, our analyses
do not consider sex-specific differences and instead should be considered an average rate of
aging for both males and females.
Endotherm datasets
        We collated mammal datasets from Lemaître et al. (55). The Lemaître et al. data were
split into males and females so we used female-only estimates because the quality of data was
usually better for females, and because averaging the male and female rates is not equivalent to a
pooled analysis (such as was done with the ectotherms). We used a subset of these data that
could be reasonably fit using the same type of model we used (i.e., Gompertz), to allow for direct
comparisons of aging rates. Thus, we   discarded mammalian datasets in which age-specific
mortality started substantially later than age of first reproduction, which requires more complex
models than the Gompertz to be fitted reliably. Human body mass was obtained from Walpole et
al. (56). We also collated avian life tables from the literature (see Data S1 for sources) and fit
Gompertz models to the data, or used parameters provided directly by researchers.
Rate of aging and longevity calculations
Datasets were analyzed using the 'basta' function from the *BaSTA* package for R (57). We
estimated the rate of aging starting at the age of maturity and going forward using a Gompertz
function (58):

$$u = e^{\beta 0 + \beta 1 * \alpha_{it}}$$

We chose this model because of its limited parameters; our wild, long-term datasets of often elusive species were sometimes too sparse to fit a model with many parameters and we wanted to facilitate comparison across as many species as possible. The age at first reproduction for each species was obtained from the literature (Data S1) and was used to determine when to start fitting the Gompertz curve. In cases where juvenile data were excluded, we set the function to estimate the relationship from age 0, since animals did not enter the sampled population until they became adults. Gompertz curves were fit to species-specific estimates for each dataset and fit was visually inspected (Fig S6). We also examined outputs from each dataset to determine that estimates from multiple chains converged. In some cases, primarily where datasets were small and recaptures infrequent, the 'basta' function returned extreme estimates. If convergence among chains or estimates could not be recovered, we re-ran analyses with longer burn-in and iteration periods.

Temperature data for Temperature Hypothesis

The approximate coordinates of each study population (Data S1) were used to extract the mean annual temperature, the maximum temperature of the warmest month, and the minimum temperature of the coldest month from the WorldClim database (*59*). Because   it was not feasible to obtain the mean temperature of the active season for each species, we assessed all three temperature estimates to determine if any had a significant impact on rate of aging and longevity.

Species-level traits for the Protective Phenotypes and Slow-fast Continuum Hypotheses

The presence of protective phenotypes, such as venom, skin toxins, shells, armor, or spines, was obtained from the literature and were classified as physical, chemical, or neither for analyses. Though there is some ambiguity about chemical protection within some amphibian groups, we deferred to previously published datasets (see Data S1 for sources). Body mass and annual fecundities from the population were included when available, or species estimates were obtained from meta-analyses (Data S1).

Phylogenetic tree

We created a supertree from recently published phylogenies of amphibians, squamates, turtles, crocodilians, mammals, and birds (*60–65*) following the topology of a recent molecular phylogeny (*66*). Consensus trees for each group were constructed from a Bayesian posterior distribution of 10,000 trees when available and these trees were then combined into a supertree without branch lengths (Fig S7). Branch lengths were estimated for the supertree using the Grafen transformation (*67*) in the *ape* package for R (*68*). To account for multiple datasets within a species, we added short polytomous branches.

Phylogenetic comparative methods

We created PGLS models in the R package *caper* (*69*) to assess ectotherm aging in general and to test our hypotheses on rate of aging (Gompertz slope parameter, ß1) and longevity (elapsed years of the adult lifespan, after the first reproduction, at which 95% of adults have died).

To compare orders within ectotherms (i.e., turtles, squamates, tuatara, crocodilians, salamanders, frogs), we first performed a PGLS and then used the *multcomp* package (*70*) to perform a Tukey's multiple comparison of means (Table S1) because the phylogenetic signal (lambda) was near 0 (<0.001). To compare ectotherms and endotherms for the Thermoregulatory Mode Hypothesis, we performed a phylogenetic ANCOVA with log body mass, group, and the

interaction between log body mass and group (i.e., ectotherm or endotherm) to assess whether the groups differed (Table 1). We did this separately for each aging measure. We also performed a PGLS each for reptiles, amphibians, birds, and mammals as described above. To see if the relationship between aging measures differed between these groups, we performed a PGLS with longevity (log-transformed), group, log body mass, and their interaction as predictors (Figure S2). Because the   phylogenetic signal was 0, we again used Tukey's multiple comparison (Table S3).

To test the Temperature Hypothesis within ectotherms, we ran PGLSs for each predictor variable (Gompertz slope and longevity) and included class (reptiles or amphibians) as a binary variable, log body mass, temperature at the population site, and a class by temperature interaction. The interaction term was included because it is possible that temperature influences reptiles and amphibians differently.

To test the Protective Phenotypes Hypothesis, protection (physical/chemical/none) was the predictor for another set of PGLSs, with log body mass as a covariate. To examine the relationships between age of first reproduction and annual fecundity and the aging estimates (Gompertz slope and longevity) to the test the Slow-Fast Continuum, we used class (reptiles or amphibians), log body mass, and the interaction between log body mass and class as predictors. For each case, we ran two separate PGLSs with rate of aging or longevity as the response variable. In any PGLS, if class, or interactions with class, was found to be significantly related to the response variable, we split the analyses by class for further investigation.

Longevity was log-scaled in order to obtain residual normality and homogeneity, and to match the allometric nature of its association with log body mass. All assumptions were met for all models. These assumptions were tested using functions from the *car* package (*71*).   Lambda was optimized with maximum likelihood for all models because branch lengths were estimated; this allows the phylogenetic signal to be estimated by transforming branch lengths.   Complete outputs for all PGLSs can be found in Tables 1, 2, S2, and S6.

Supplementary Text

Species-level trait data collection

The age at maturity for each species was obtained from the literature (Data S1) and was used to determine when to start fitting the Gompertz curve. There is a lot of variation because age at first reproduction in many reptiles and amphibians is better estimated by size than age. If a range was given, the median age was used, and if males and females differed, the one with the higher age at maturity was used. For amphibians, where age of maturity often corresponds to metamorphosis from a larval aquatic stage to a terrestrial or semi-aquatic reproductive stage, we treated each dataset on a case-by-case basis. Amphibian datasets with no juveniles had a truncation age of 0, but this corresponds to the age of metamorphosis. For some species for which species-level data were not available, we used conspecific members of the same genus (all sources can be found in Data S1).

Clutch size and clutches per year were obtained from meta-analyses, such as Allen et al. (*72*), when possible, and from species-specific papers when necessary. These values were multiplied to obtain annual fecundity values. The number of c   lutches per year was not available for most amphibian species. In amphibians, annual breeding probability may vary among populations of the same species (*73*, *74*) and over time within a population (*75*). As this information is lacking in most amphibian populations, we assumed that females produce one egg

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clutch per year with constant size, an assumption that has been previously made in demographic models for amphibians (see for instance *72* for frogs and salamanders).
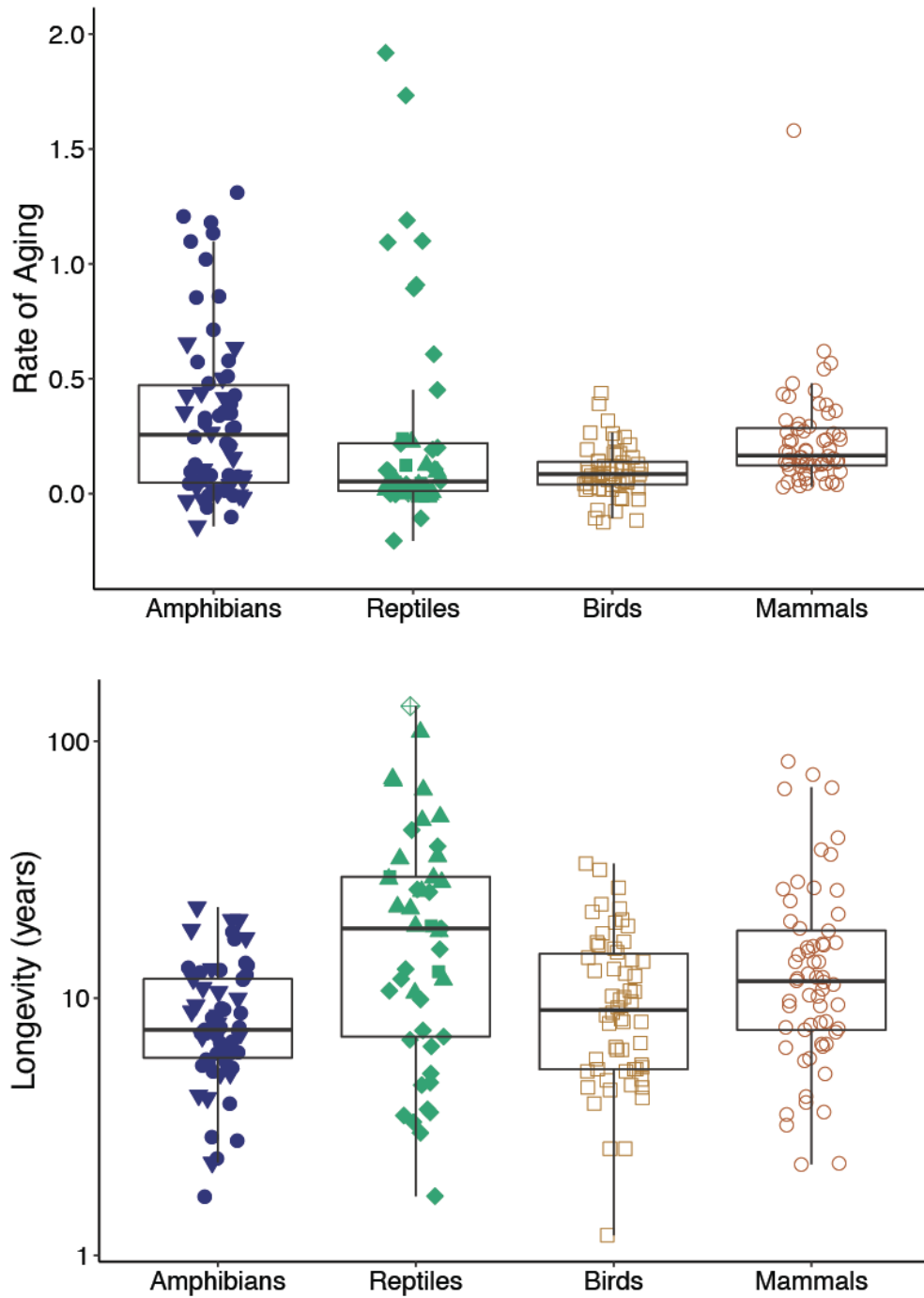
Fig. S1.

Distribution of rates of aging and longevities in amphibians, reptiles, birds, and mammals.
The rate of aging here is the mortality slope derived from a Gompertz model. Longevity is the
years since age at first reproduction at which 95% of the individuals in a population have died.
The data are not mass or phylogeny-corrected. Symbols correspond with those in other figures.
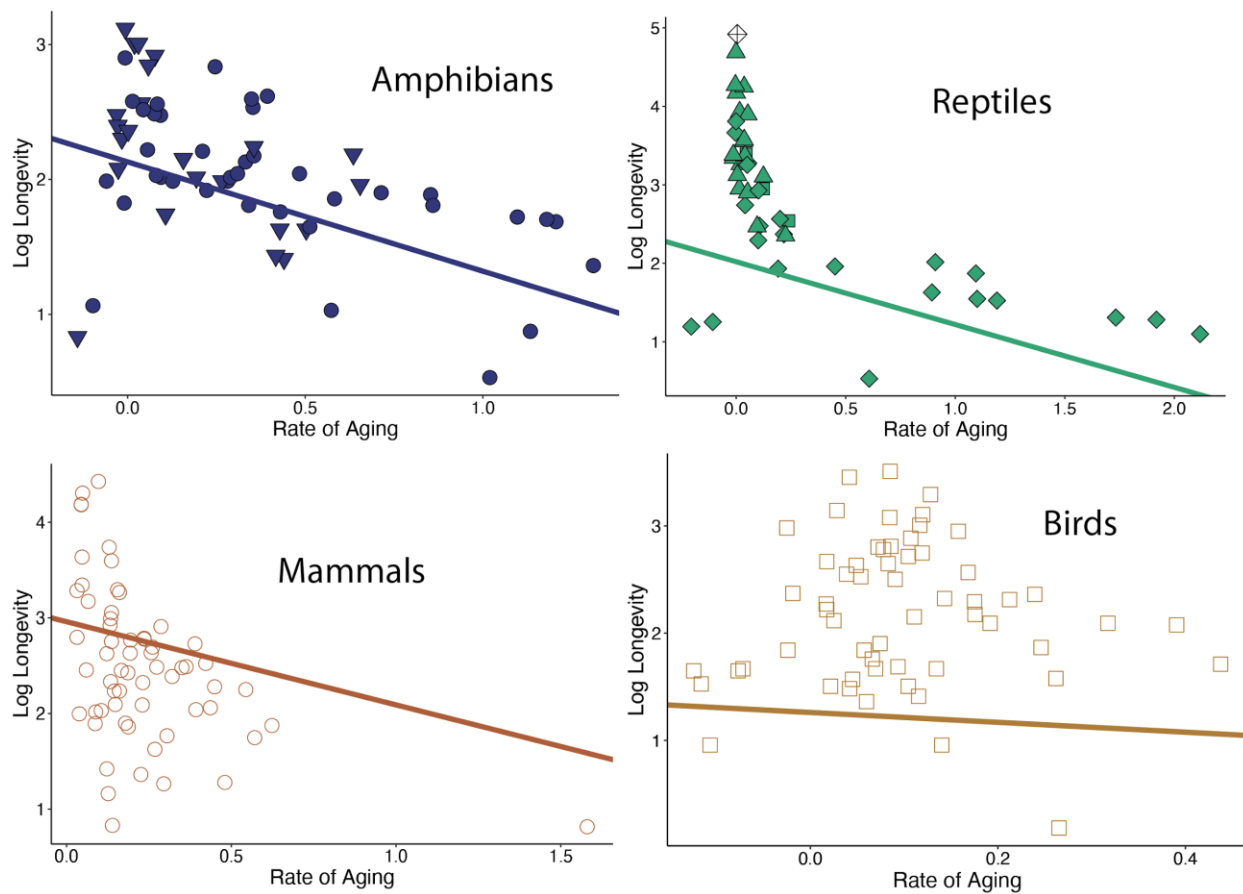
Fig. S2.

Relationship between rate of aging and longevity for amphibians, reptiles, mammals, and birds. Regression lines result from the PGLS models and are corrected for phylogeny and body mass. Symbols used here are the same as in other figures.
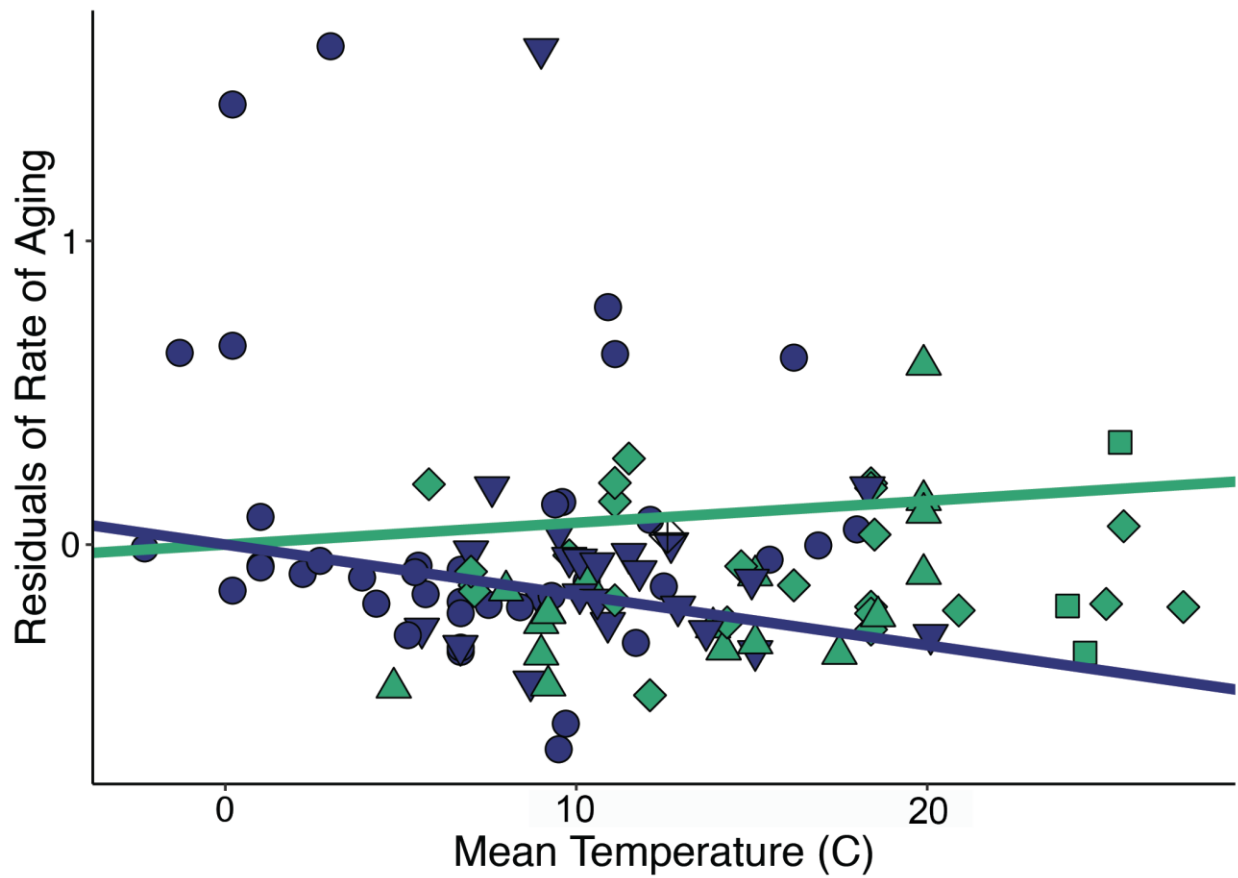
Fig. S3.

Relationship between mean annual temperature and rate of aging. There was a near significant negative trend in mean temperature and rate of aging, measured as the Gompertz mortality slope (p=0.08, Table 2), with a significant class by temperature interaction. Regression lines are derived from the PGLS model in Table 2 for the significant class by temperature interaction. Symbols and colors used here are the same as in other figures, with green representing reptiles and purple representing amphibians.
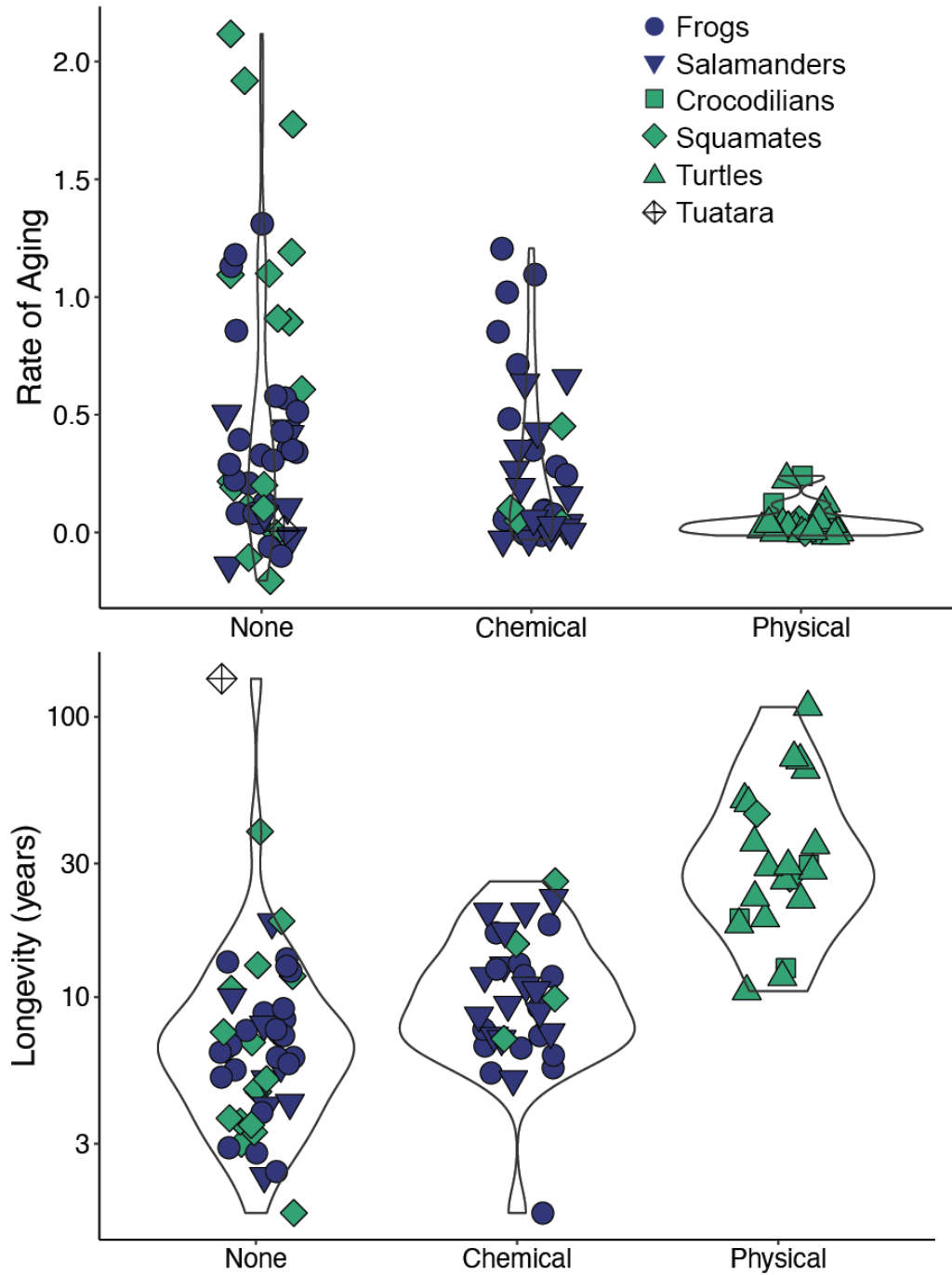
Fig. S4.
Measures of rate of aging and longevity by protective phenotypes. Species with physical protective phenotypes, such as shells or armor, have slower rates of aging and longer longevities than those without. Points are colored as in other figures, with green representing reptiles and purple representing amphibians.
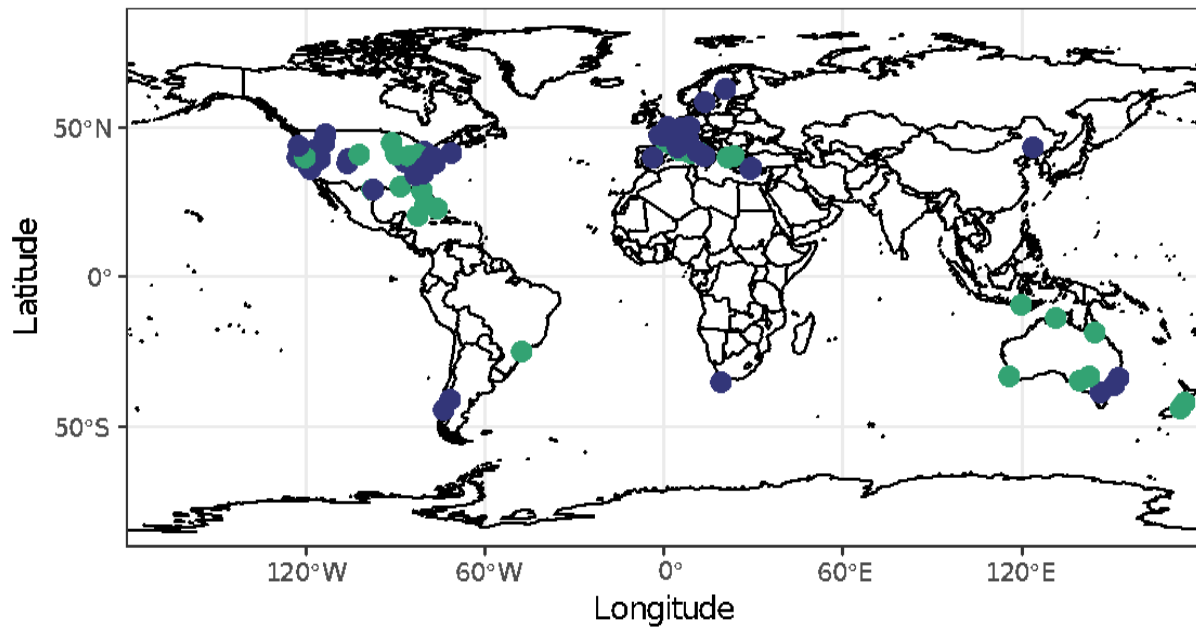
Fig S5.
Distribution map of datasets. Green dots represent reptile datasets and purple dots represent amphibian datasets. The authors recognize the Western bias inherent in this collection of datasets and want to stress this bias as a reminder of the systemic issues with science and science funding that lead to many areas being under-utilized or under-represented in long-term research.
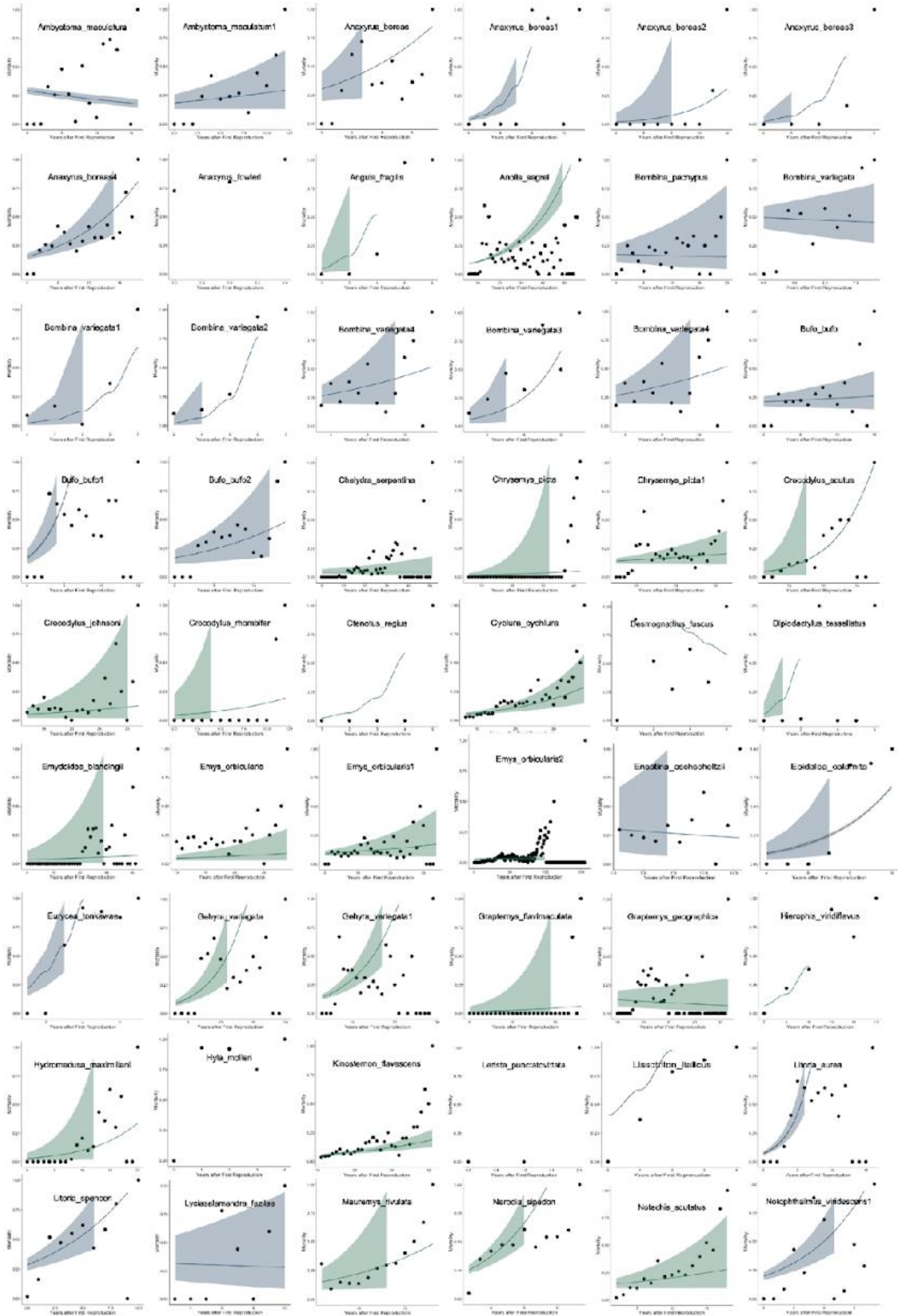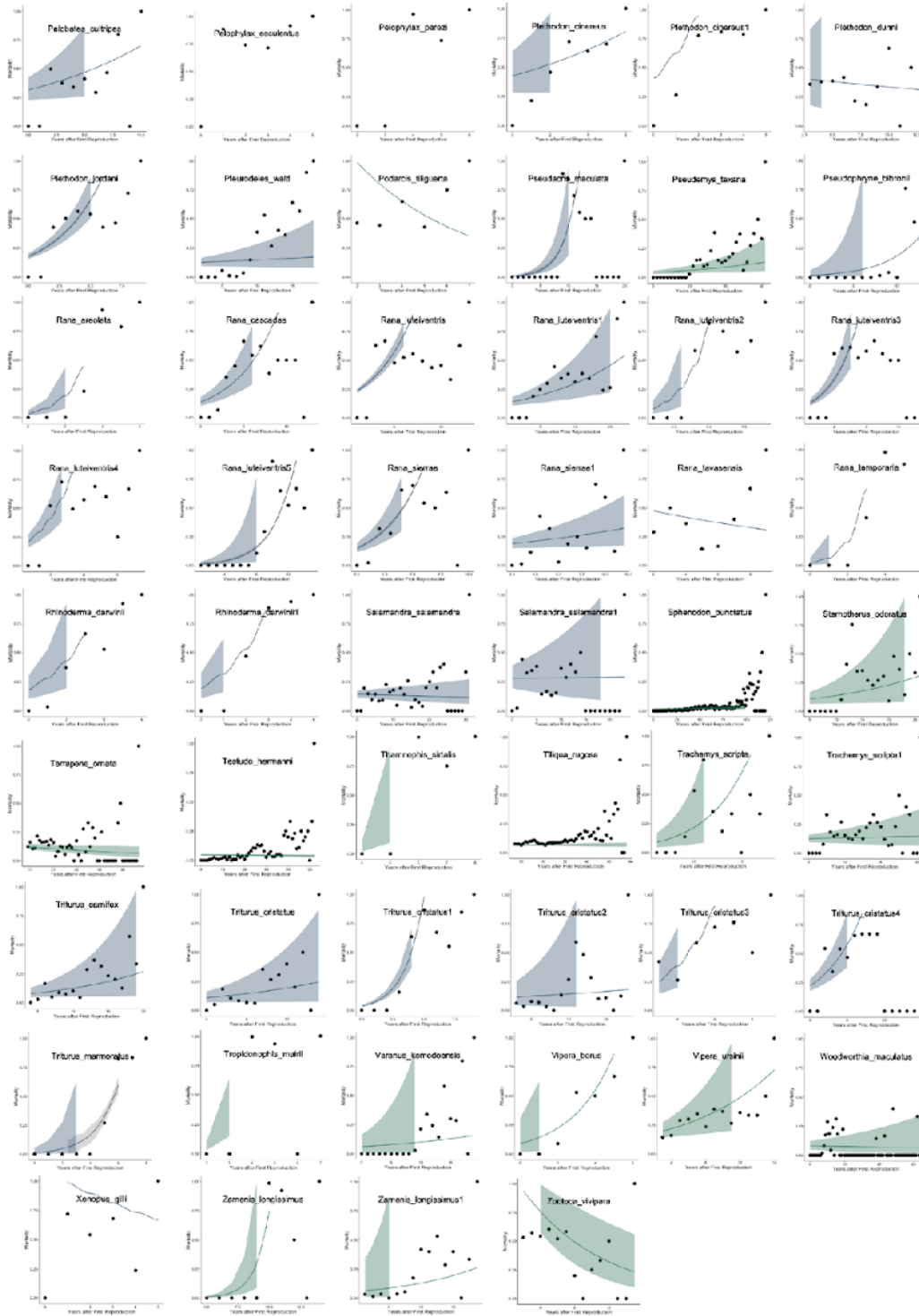
Fig S6.
Fit of model estimates and Gompertz curves for each dataset. All axes are the same (years after first reproduction on the X, mortality on the Y). Shaded areas represent the 95% credible intervals, with green representing reptile species and purple representing amphibian species.
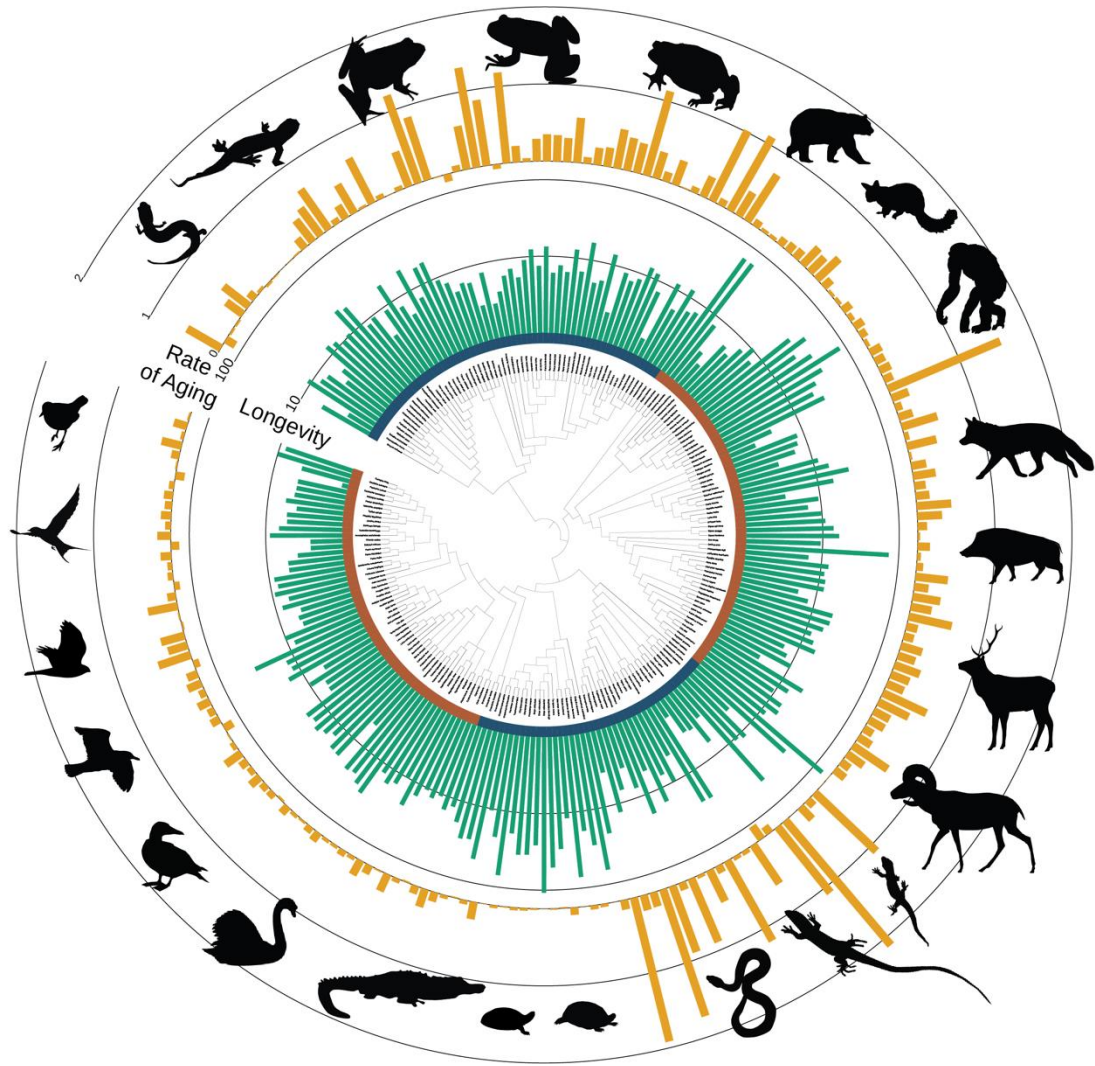
Fig S7.
Supertree of all endothermic and ectothermic species included in this analysis. Branch lengths are not scaled. The red in the inner circle represents endotherms and blue represents ectotherms, as throughout the paper. Green bars are longevity estimates and orange bars are the aging rates. Silhouettes from Phylopic.org.

Table S1. Descriptive summary of ectotherm aging parameters used in this study by group. Note that values are not corrected for body mass or phylogeny. See Data S1 for species-specific values.

	# of Species	# of Datasets	Average Rate of Aging (SE)	SD Rate of Aging	Rate of Aging Range	Average Longevity (SE)	SD Longevity	Range Longevity
Amphibians	**38**	**62**	**0.33 (0.05)**	**0.36**		**8.99 (0.60)**	**4.72**	
Frogs	23	39	0.41 (0.06)	0.40	-0.10 – 1.31	8.17 (0.62)	3.85	1.7 – 18.2
Salamanders	15	23	0.18 (0.05)	0.23	-0.14 - 0.65	10.37 (1.20)	5.74	2.3 – 22.6
Reptiles	**39**	**45**	**0.31 (0.08)**	**0.55**		**26.19 (4.16)**	**27.90**	
Crocodilians	3	3	0.14 (0.06)	0.10	0.04 - 0.24	20.5 (4.96)	8.59	12.7 – 29.7
Squamates	21	23	0.55 (0.14)	0.69	-0.20 - 2.12	12.06 (2.44)	11.72	1.7 – 45.1
Turtles	14	18	0.04 (0.01)	0.06	-0.01 – 0.23	39.04 (6.04)	25.64	10.5 – 108.5
Tuatara	1	1	0.005	----	----	136.8	----	----

Table S2.

Estimates of Tukey's post-hoc pair-wise comparison of differences in rates of aging and mean log(longevity) among ectotherm orders, controlling for body size and phylogeny. Bolded estimates had p-values < 0.05. Standard errors are in parentheses. Rate of aging (β_1) is the response variable above the diagonal and longevity (on a log scale) is the response variable below the diagonal.

	Frogs	Crocodilians	Squamates	Tuatara	Turtles	Salamanders
Frogs		-0.03 (0.32)	0.17 (0.11)	-0.31 (0.43)	-0.26 (0.15)	-0.28 (0.11)
Crocodilians	-0.11 (0.46)		0.20 (0.31)	-0.28 (0.50)	-0.23 (0.28)	0.25 (0.35)
Squamates	0.01 (0.16)	0.12 (0.45)		-0.48 (0.43)	**-0.43 (0.15)**	**-0.44 (0.14)**
Tuatara	**2.51 (0.62)**	**2.62 (0.72)**	**2.50 (0.62)**		0.05 (0.43)	0.03 (0.44)
Turtles	**0.99 (0.22)**	1.09 (0.41)	**0.97 (0.22)**	-1.52 (0.62)		-0.02 (0.18)
Salamanders	0.40 (0.17)	0.50 (0.50)	0.38 (0.20)	**-2.11 (0.64)**	-0.59 (0.27)	

Table S3. Estimates of Tukey's post-hoc pair-wise comparison of differences in mean rates of aging and longevity (on a log scale) among tetrapod classes, controlling for body size.
Bolded estimates had significant p-values. Standard errors are in parentheses.

	Amphibians	Reptiles	Birds
Reptiles	0.21 (0.18)		
Birds	**-0.92 (0.19)**	**1.13 (0.17)**	
Mammals	**-0.51 (0.19)**	**0.72 (0.17)**	0.41 (0.18)

Table S4. Statistical output for ectotherm PGLSs showing output of all predictor variables for the Temperature Hypotheses, using maximum temperature of the warmest month and minimum temperature of the coldest month. Class is a factor with two levels: reptile and amphibian.

PGLS Model	Df	Sum Sq	Mean Sq	F value	Est	P-value
Maximum Temperature						
Rate of aging (Adj R^2 = 0.05, λ=0.30)						
Class	1	0.01	0.01	0.01	-1.10	0.09
Max temp	1	0.64	0.64	2.73	-0.0002	**0.88**
Class × max temp	1	1.01	1.01	4.32	0.004	0.04
Log mass	1	0.66	0.66	2.83	-0.05	0.05
Residuals	102	23.95	0.23			
Log longevity (Adj R^2 = 0.19, λ=0.74)						
Class	1	0.69	0.69	0.55	0.97	0.51
Max temp	1	8.98	8.98	7.12	-0.002	**0.14**
Class × max temp	1	0.67	0.67	0.53	-0.002	0.47
Log mass	1	26.43	26.43	20.96	0.18	<0.001
Residuals	102	126.56	1.24			
Minimum Temperature						
Rate of aging (Adj R^2 = 0.05, λ=0)						
Class	1	0.009	0.009	0.04	0.19	0.10
Min temp	1	0.002	0.002	0.01	-0.001	**0.17**
Class × min temp	1	0.72	0.72	3.79	0.002	0.05
Log mass	1	1.01	1.01	5.30	-0.06	0.007
Residuals	102	19.51	0.19			
Log longevity (Adj R^2 = 0.13, λ=0.59)						
Class	1	0.77	0.77	0.81	0.19	0.85
Min temp	1	0.01	0.01	0.01	0.0002	**0.87**
Class × min temp	1	0.33	0.33	0.35	-0.001	0.55
Log mass	1	17.47	17.47	18.51	0.18	<0.001
Residuals	102					

Table S5.
Estimates of Tukey's post-hoc pair-wise comparison of means with protection as the predictor. Bolded estimates had significant p-values < 0.05. Standard errors are in parentheses. Rate of aging (β_1) is the response variable above the diagonal and longevity (on a log scale) is the response variable below the diagonal.

	None	Chemical	Physical
None		**0.25 (0.10)**	**-0.57 (0.16)**
Chemical	-0.33 (0.16)		-0.32 (0.15)
Physical	**1.21 (0.25)**	**0.88 (0.24)**	

Table S6. Statistical output for PGLSs split by class (reptiles and amphibians) to test the Slow-fast Continuum Hypothesis. Classes were split when class was found to be a significant predictor. P-values <0.05 are bolded.

Reptiles	Df	Sum Sq	Mean Sq	F value	Est	P-value
Rate of aging (Adj R^2 =0.18)						
Log age of repro	1	2.32	2.32	4.23	-0.31	**0.05**
Log annual fecundity	1	0.02	0.02	0.04	0.03	0.78
Log mass	1	4.77	4.77	8.70	-0.005	0.92
Residuals	41	22.50	0.55			
Amphibians						
Rate of aging (Adj R^2 =0.26)						
Log age of repro	1	0.66	0.66	1.53	-0.19	0.22
Log annual fecundity	1	3.16	3.16	7.37	0.10	**0.01**
Log mass	1	1.75	1.75	4.08	-0.08	0.18
Residuals	55	23.61	0.43			

Table S7. Statistical output of PGLSs to test if annual fecundity and age at first reproduction vary by class (reptiles or amphibians) before testing the Slow-Fast Continuum Hypothesis

	Df	Sum Sq	Mean Sq	F value	Est	P-value
Age at First Reproduction (Adj R^2 =0.28, $\lfloor$=0.57)						
Class	1	19.87	19.87	2.77	1.54	**0.55**
Log mass	1	281.64	281.64	39.25	0.69	<0.001
Residuals	103	739.11	7.18			
Annual Fecundity (Adj R^2 =0.04, $\lfloor$=0.99)						
Class	1	1620041	1620041	0.06	-2313.10	**0.72**
Log mass	1	190491480	190491480	6.69	179.64	0.01
Residuals	101	2874266507	28458084			

Data S1. (separate file)

A summary of all datasets including species and population-level traits used in the analyses, their sources, and the rate of aging and longevity calculated in this paper.