



Lizards from warm and declining populations are born with extremely short telomeres

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Aging is the price to pay for acquiring and processing energy through cellular activity and life history productivity. Climate warming can exacerbate the inherent pace of aging, as illustrated by a faster erosion of protective telomere DNA sequences. This biomarker integrates individual pace of life and parental effects through the germline, but whether intra- and intergenerational telomere dynamics underlies population trends remains an open question. Here, we investigated the covariation between life history, telomere length (TL), and extinction risk among three age classes in a cold-adapted ectotherm (*Zootoca vivipara*) facing warming-induced extirpations in its distribution limits. TL followed the same threshold relationships with population extinction risk at birth, maturity, and adulthood, suggesting intergenerational accumulation of accelerated aging rate in declining populations. In dwindling populations, most neonates inherited already short telomeres, suggesting they were born physiologically old and unlikely to reach recruitment. At adulthood, TL further explained females' reproductive performance, switching from an index of individual quality in stable populations to a biomarker of reproductive costs in those close to extirpation. We compiled these results to propose the aging loop hypothesis and conceptualize how climate-driven telomere shortening in ectotherms may accumulate across generations and generate tipping points before local extirpation.

aging | ectotherms | population extinction | telomeres | life-history tradeoffs

Biodiversity is undergoing a global crisis, highlighted by the alarming pace of species loss worldwide (1, 2). This trend is accelerating, especially due to pervasive effects of climate warming on extinction risks in range-limited or cold-adapted species (3–5). A current issue is our virtual blindness about the nature and generality of the underlying mechanisms connecting warmer climates to individual life history, and early warning signals of warming-induced extinctions (6, 7). All living creatures eventually die but the time separating birth or first reproduction from death can be altered by climate warming and heat stress because low body temperatures generally benefit lifespan in both endothermic and ectothermic animals (8–10). In terrestrial ectotherms, climate warming can also constrain activity patterns (4) and significantly increase maintenance costs (11, 12) as well as the risks of heat stress (13). Together, these may eventually alter fundamental life history tradeoffs and a switch to a “live fast, die young” life history strategy along the slow–fast continuum (9, 10, 14).

These demographic effects of global warming in terrestrial ectotherms should be driven by physiological mechanisms whereby higher temperatures cause an acceleration of growth and reproduction, induce whole-organism oxidative stress, and eventually hasten the rate of aging (9, 15). Still, how physiological changes in aging rates and longevity will trade with individual performance in the long run and push populations at the warm edge of the range distribution on the verge of extinction remain unclear because ectotherms can display a variety of buffering mechanisms to compensate for these potential changes in lifespan (16). Telomeres, the protective terminal DNA sequences of linear chromosomes, represent promising biological markers to investigate these physiological mechanisms of warming-induced extinctions because they may be internal regulators of life history trajectories (17). Telomere length (TL) normally shortens at each cell division, and, once a length threshold is crossed, end-replication problems lead to cell apoptosis or genome instability at cellular and tissue levels (18). The rate of telomere erosion integrates both the inherent aging process (i.e., standard cell division rates and associated damage accumulation in a given tissue) and also additional, cumulative effects of stressors, including those due to heat stress (19–21). Eventually, TL and/or telomere attrition rates in somatic tissue predict lifespan and lifetime reproductive success among and within many species (22–24).

Telomere erosion can also be impacted by environmental stressors in the germline, explaining both immediate fitness costs and transgenerational effects (25). Multiple biotic

Significance

This study unraveled the impacts of accelerated aging as a corollary of climate-driven population decline. We found a transgenerational accumulation of telomere shortening implying that offspring were already born “old.” We suggest that this process may exacerbate across generations, leading to an aging loop in the population. This model posits that telomere dynamics should represent a critical and promising molecular biomarker of population extirpation as well as a way to test the *in situ* efficiency of conservation decisions.

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The authors declare no competing interest.

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and abiotic factors may alter gamete TL (26), which may condition environmental influences on TL heritability (27). The TL is normally reset by telomerase activity in germ cells, zygotes, and early life (18), but deleterious parental effects such as abnormally high and stressful developmental temperatures may either directly favor telomere shortening and/or indirectly affect telomerase repair functions in early life (28, 29). However, impacts of external temperature on telomere dynamics during embryonic life in juvenile ectotherms are not that straightforward, as shown by lacking effect of incubation temperature in Atlantic salmon (30) or thermal effects conditioned by pollutant exposure in American alligators (31).

An open question is whether warming-induced acceleration of intragenerational pace of life and population-extinction risks further combines with intergenerational patterns of TL erosion. Testing this demands concurrent measures of TL at different life stages, in parents and offspring from natural populations chronically exposed to a range of climate conditions, including weather extremes conducive to long-term population decline. We thus used a well-known geographic gradient of climate-induced extinction risk across the range of the cold-adapted common lizard (*Zootoca vivipara*), which is now exposed to climate warming and drying (32) and trapped around mountain ranges in its meridional Western European distribution (southern France and northern Spain). Natural populations experience repeated summer heat waves and dry spells in lowland habitats that have multiple damaging impacts, including a faster pace of life, loss of lifetime reproductive fitness, loss of dispersal, and higher inbreeding levels that combine to cause extinctions in some populations (4, 14, 33, 34). We previously established that shorter TL of yearlings in warmer habitats may precede population extinction but this did not allow us to separate potential intragenerational and intergenerational mechanisms (35).

In this study, we examined whether and how shorter TL could result from maternal transmission. For more than 10 y, we monitored 22 wild populations of common lizards in France's Massif Central mountain along a geographic and altitudinal gradient ranging from cold highland to warm lowland margins (*SI Appendix, Fig. S1*). These populations are spread along a continuum of

extinction risk encompassing recent abundance changes, thermal conditions, altitude, and life history strategies, as summarized by an extinction risk score (main axis of a principal component analysis [PC1] score, *SI Appendix, Fig. S2*). We explored the covariation between population state and TL in 126 adult females and their progeny (231 offspring) from nine representative populations (seven stable or expanding and two declining) to complement TL data collected in yearlings 2 y before from the same populations and from a further population potentially extirpated since then. We further investigated how thermal conditions during pregnancy (i.e., basking opportunity), maternal traits, and offspring characteristics could explain variation in lizard TL at birth and further predict female reproductive performance at

Results

Breakpoints in TL along Extinction Risk. We found that TL follows the same threshold relationship along the PC1 score among newborn, yearling, and adult females, attaining stunted states concomitant to greater risks of extinction in the declining populations (Fig. 1). Breaking points before noticeable changes in TL coincided with the demographic threshold of population extinction and were qualitatively similar across the three age classes (*SI Appendix, Fig. S3*).

Offspring TLs Are Already Shorter at Birth in Collapsing Populations. Offspring experienced abnormally short TL in the two natural populations close to demographic collapse (*SI Appendix, Table S1*). Based on previous evidence linking juvenile mortality risk to early life TL in this species (23), we used the range of TL values in yearlings that did not survive to obtain an estimation of TL thresholds predicting high death risks and survival chances (Fig. 2*A*). Below the lower threshold of this TL range, only a very low proportion of juveniles (7%) in declining populations is expected to survive and reach 1 y of age and recruitment, corresponding to the lower range seen in interannual studies of multiple birth cohorts, marked and monitored in a stable population (36). Inversely, this calculation predicts a way higher survival chance in stable populations (73%), a value getting

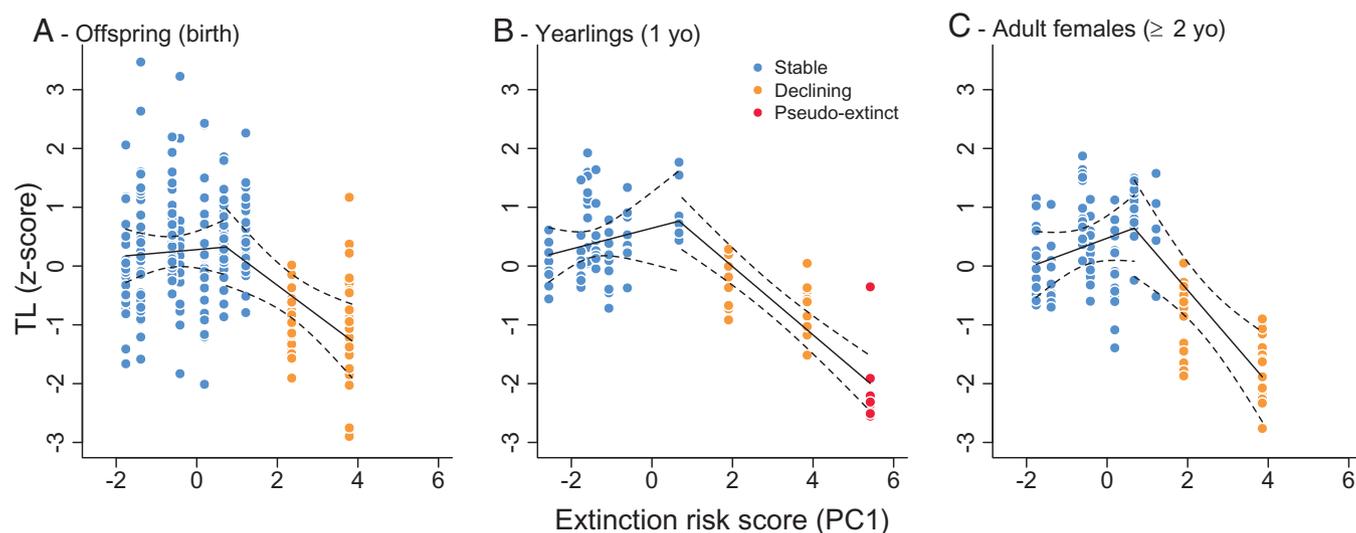


Fig. 1. Threshold relationships between TL of common lizards and a composite score of the extinction risk of their population (coordinate along the main axis of a PCA, *SI Appendix, Fig. S2*; high values of PC1 indicate population collapse, high night-time temperatures, low altitude, and a fast pace of life). Breakpoint occurs for qualitatively similar tipping points of extinction risk scores in (A) offspring (breakpoint: 0.73, CI 95%: -0.69 to 2.15), (B) yearlings (breakpoint: 0.67, CI 95%: -0.60 to 1.95), and (C) adult females (breakpoint: 0.67, CI 95%: -0.28 to 1.63). Data for yearlings were obtained from an earlier study (35) and included one population presumably extinct between 2015 and 2017–2018 (personal observation). All TL data were centered and standardized by a z-score transformation to ease visualization (raw distribution available at *SI Appendix, Fig. S3*). Best regression lines and confidence bands are displayed with individual data points.

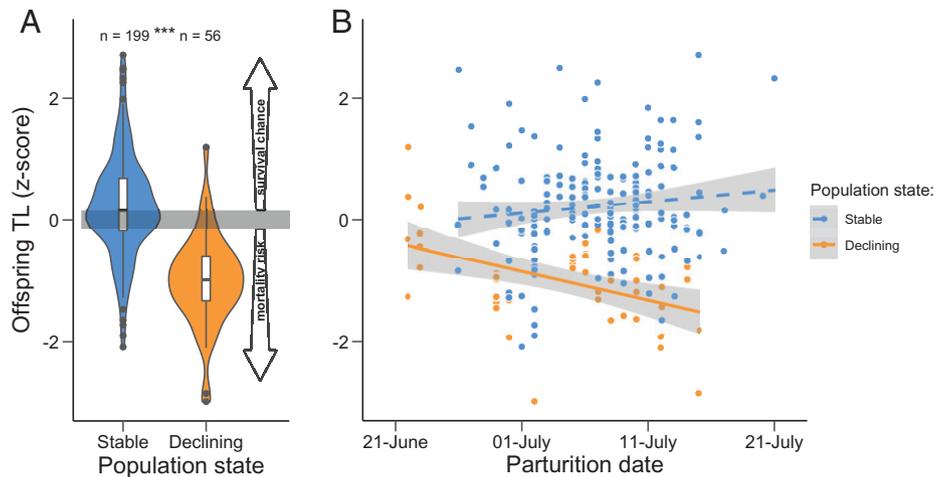


Fig. 2. Offspring common lizards are born “old” in declining populations. (A) TL is already short at birth in offspring from declining populations compared to those from stable ones. Based on a previously established range of TL values for thriving young lizards (23), we estimated the threshold TL for early life survival (gray bar) and predicted that 90% of offspring should not even attain recruitment. (B) Offspring TL is negatively correlated to parturition date in declining populations (orange symbols, solid line, $P = 0.003$) but not in stable ones (blue symbols, dashed line, $P = 0.251$).

into the upper range seen in interannual studies of a stable population (36). Offspring TL was also positively correlated with mother TL (*SI Appendix, Table S1*) and covaried interactively with size-adjusted parturition date and population state (*SI Appendix, Table S1*). There was no effect of parturition date in stable populations as opposed to a negative relationship between TL and parturition date among offspring from declining populations (Fig. 2B). Other offspring traits (sex or juvenile size) or maternal characteristics (mother size or basking treatment) had no influence on TL variation at birth (*SI Appendix, Table S2*).

We partitioned variance in a random model on offspring TL among populations ($\sigma^2_{\text{population}} = 0.36$), among families within the same population ($\sigma^2_{\text{family}} = 0.31$) and within families ($\sigma^2_{\text{residuals}} = 0.40$). A full-sib analysis among offspring from the same family under the strong assumption of no maternal effect suggested high levels of TL broad-sense heritability across all populations ($H^2 = 0.57$). Accounting for the variance explained by fixed effects of the final mixed model (*SI Appendix, Tables S1 and S2*), heritability estimation slightly decreased to $H^2 = 0.53$.

Adult TL Further Predicts Reproductive Performance. In pregnant females, population trajectory and TL interactively explained variation in fitness-related reproductive traits (*SI Appendix, Tables S1 and S3*), supporting previously observed reproductive senescence in warming environments for this species (37). Specifically, reproductive investment (total fecundity corrected for body size) increased with the TL of pregnant females in stable populations (Fig. 3A), whereas it decreased with TL in declining populations (Fig. 3B). Instead, no relationship occurs between litter success (the proportion of viable offspring) and TL of pregnant females in stable populations (Fig. 3C), while litter success dropped in collapsing populations together with shorter TL of pregnant females (Fig. 3D). These findings shed light on the classical relationship between TL and fitness (22, 38) by suggesting that fitness costs of shorter TL for reproduction are more likely to occur under harsh environmental conditions.

Discussion

In ectotherms such as the common lizard, climate warming causes an accelerated life history, which translates into faster body growth, sexual maturation earlier in life, and parturition sooner in the year in both natural or captive populations (14, 39–41).

Despite the short-term fitness benefits (40), this more productive life history induces demographic costs on the long run. This is illustrated by a drop in the relative abundance of lizards since 2005 in natural populations and the loss of lifetime reproductive fitness due to a shorter longevity of lizards in populations exposed to warmer summer weather (14). Supporting the climate-induced shift to extirpation, we witnessed over the last decade the pseudoextinction of a wild population in the warmest location (Mont Caroux, Fig. 1B), in which yearlings were very large but exhibited completely eroded TL in 2015 (35) and could not be sampled in subsequent years (including 2017) despite our efforts. The causal role of environmental temperatures, potentially together with drought (23), in triggering these changes in life history strategies and leading to pseudoextinctions is now well documented in this cold-adapted lizard and might be a general pattern in other ectotherms (9).

Offspring Are Being Born Already “Old.” Our results also corroborate previously established impacts of environmental temperatures on telomere erosion in germline and somatic tissue of nonavian reptiles (29, 42, 43) and more generally in ectotherms (44). The nonlinear relationships between TL and population status, which combines information on population decline, environmental temperatures, and life history traits, further conform to the conclusions of a recent review of aging mechanisms in ectotherms (9). A steep decline of TL characterized the transition from stable to declining populations in all age classes, probably because populations at warmer locations are more regularly exposed to heat waves leading to stress reactions, reactive oxygen species production, and telomere shortening (15). Yet, one of the most striking results from our study unravels that offspring from declining populations already showed eroded telomeres at birth with a geographic variability qualitatively matching the one observed in yearlings and adults (*SI Appendix, Fig. S3*). Shorter TL could have resulted from inheritance together with additional attrition or altered resetting by telomerase during embryonic development as commonly hypothesized (45, 46). Telomerase activity closely explains telomere dynamics in lizards (47), so its sensitivity to climatic conditions during embryonic life is likely crucial and deserves specific attention. We cannot draw any firm conclusions regarding the role of early developmental and paternal lineage effects since TL was not sampled in the germline of adults and in fathers and also because we lack information about TL

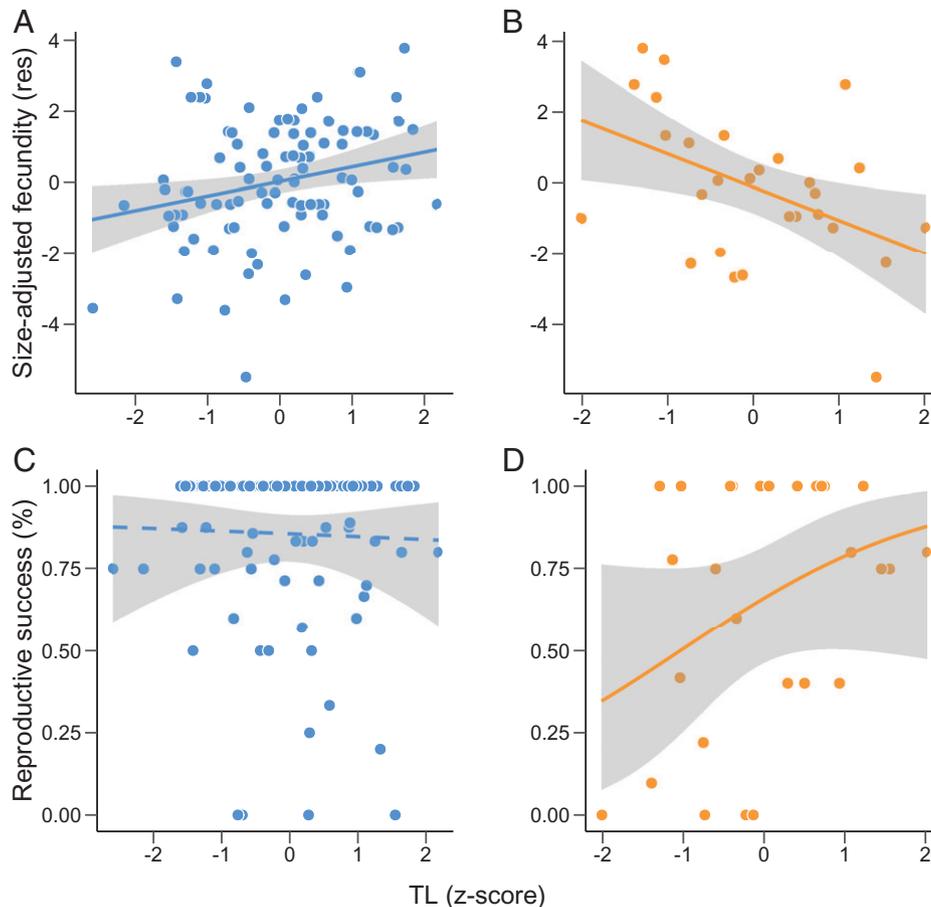


Fig. 3. Context-dependent relationships between TL in pregnant female common lizards and their reproductive output are obvious from a post hoc comparison of stable and declining populations. (A and B) Total fecundity adjusted for female SVL increases slightly with TL in stable populations ($P = 0.020$) but decreases with TL in declining populations ($P = 0.012$). (C and D) Litter success correlates positively with TL only in declining populations ($P = 0.007$). Figures represent raw data points, best regression lines, and 95% confidence bands.

prior to birth in offspring. Yet, the lack of effects from the manipulation of maternal basking time on offspring TL at birth suggests a relatively low influence of daily temperature on embryonic development (30). In further support of minor prenatal environmental effects, offspring from declining populations and born earlier in the year exhibited longer TL than those born 2 to 3 weeks later despite the fact that their mothers spent more time in their natural habitat than in standard *ad libitum* laboratory conditions. This implies that the proportion of developmental time spent within the natural habitat did not explain why offspring displayed shorter TL at birth. Instead, we propose that offspring TL variation falls along a continuum from early to late phenology indicated by differences in female quality, given the recurrent finding that reproduction follows a “sooner is better” pattern in lizards living in seasonal environments (29). These observations coupled with relatively high heritability estimations from the full-sib analysis and a strong mother–offspring correlation for TL imply that the shorter telomeres in offspring were more likely inherited at conception through parental gametes instead of being reset after fecundation and shortened during early embryonic life.

Consequences of Shorter TL. In general, population variability in TL may reflect either differences in initial TL at conception and/or birth, differences in TL dynamics with age (e.g., increased telomere erosion or reduced telomere restoration), selective disappearance of individuals with different TL, or biases due to the age structure of the population (48). In lizards, TL relates to age following quadratic or positive relationships (47), so shorter TL in

adult females from declining populations may reflect recent changes in age distribution biased to a majority of young adults (2 y old). Importantly, this alteration of telomere dynamics may induce greater annual mortality risks of lizards (49), including in this species (23) and the drop of female reproductive effort (37), in line with general evidence linking TL with individual quality across vertebrates (22, 38). Reproductive success is also known to positively correlate with TL in some species (50) and specifically to the capacity of females to mount antioxidant shielding among our sampled lizard populations (51). This relationship between TL and reproduction involves both TL as a biomarker of individual quality to produce offspring (positive association between TL and reproduction) and TL as a mediator of reproductive costs (positive association between reproduction and TL shortening between species (52). Here, we found context-specific associations between TL of pregnant females measured during midgestation and their fecundity or reproductive success with striking difference between stable and declining populations. In declining populations, TL variation among females within the population was positively correlated with reproductive success but negatively with reproductive effort, whereas it was positively correlated with reproductive effort in stable populations. This intriguing result suggests a threshold transition from TL as a biomarker of female quality in stable populations to a cost of reproduction under harsh conditions. Accordingly, experimental increase of TL with drugs (53) or engineering of maternal reproductive effort (54) should help investigate if and when TL may switch from an index to a driver of reproductive costs.

The Aging Loop Hypothesis. We propose a conceptual mechanistic model of an aging loop linking climate warming with physiological stress and population demography in order to explain how and why population extirpation may follow a vicious circle dynamic in this species (Fig. 4). Central to this concept is telomere dynamics, an integrative mediator linking chronic climate stress to pace of life strategies within and between generations, eventually predicting the loss of reproductive individuals and demographic collapse (Fig. 4). Local extinctions are often preceded by tipping points and early warning signals that precede extinction in deteriorating environments such as dynamic phenomena related to demographic variance and density dependence, but those are often difficult to detect without a detailed time series of population dynamics (55). At a lower organizational level, cell life span and organismal functioning and viability also follow threshold effects related to telomere shortening (18). As observed here, most individuals from collapsing populations exhibited TL below the range measured in thriving individuals even at birth, which implied exacerbated mortality risks in the warmest, low altitude populations. Associated drops in population resilience capacity to endure any additional and stochastic events will push it to the edge of extinction before its complete collapse.

Conclusion. Applying similar themes from biomedical research in humans, ecological studies have focused on key aspects of telomere biology regarding age and life history effects, inheritance, and environmental factors accelerating telomere erosion (19, 56). In line with other recent studies, we believe the time has come to take into account and model the roles of telomere dynamics in the conservation biology of wild populations (35, 57–59). Such

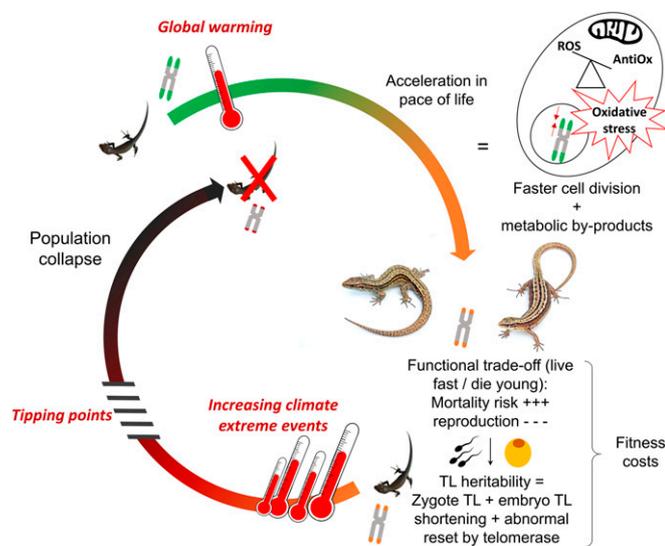


Fig. 4. The “aging loop hypothesis” for climate-induced population decline in the common lizard. We propose that climate warming increases the pace of life and rate of physiological aging, which eventually leads to a vicious circle through accumulation of shorter telomeres in the population. Telomeres get short sooner because warmer temperatures induce faster cell division and greater oxidative stress and/or because of an increased frequency of stressful weather events. Across generations, shorter telomeres may accumulate in the populations if telomere length is heritable and because of the shortening of telomeres through the germline. This pattern can result from either direct impacts of elevated physiological rates on telomere attrition or indirect alteration of normal resetting processes through telomerase activity during embryonic development. Greater telomere loss may then predict lower reproductive fitness that amplifies across generations. Shorter TL in early life is associated with lower survival rate in this species (23) implying that offspring are born physiologically too “old” to survive to adulthood. Eventually, after a given tipping point, climate warming should bring down the number of recruits together with the resilience of the population. Abbreviations: ROS, reactive oxygen species; AntiOx, antioxidant defenses.

mechanistic comprehension might be an essential step to understand the proximate causes of demographic risks and to promote efficient conservation measures. In particular, TL may represent a promising tool to outpace tipping points. First, investigating TL dynamics in wild populations without extensive background demographic data should allow for the rapid identification of the populations most at risk. Second, TL may also constitute a biomarker to test *in situ* the impacts of restoration programs or reintroduction strategies without necessarily relying on detailed, time-consuming demographic studies. Thus, our correlative study of extinction mechanisms in a single species provides a stepping stone for global analyses of the relationship between climate warming, pace of life, and accelerated TL erosion along with the development of TL as a biomarker to anticipate and potentially prevent ongoing population extinctions.

Materials and Methods

Field Population Survey. Each population was visited during 1 d to collect approximately 24 pregnant females, 10 adult males, and 20 nonreproductive yearlings in June 2017 and 2018. Males and yearlings were measured for body mass and body size (snout-to-vent length, SVL) by the same operator and released at the end of the day. Females were brought back to a field laboratory and released after parturition (see below). We used the total time spent performing captures in the field, the number of people involved, and the total surface of the population to estimate an index of lizard abundance (in number of lizards·ha⁻¹·h⁻¹·person⁻¹). The relative change in lizard abundance since 2005 provides an estimate of demographic trends in recent years (35). Since 2015, populations were also equipped with data loggers for a month at the peak of annual activity to characterize thermal conditions. Each year, we recorded temperature every hour using three loggers in each population (iButtons, Maxim Integrated Products, ±0.0625 °C protected in shelters) placed within vegetation (ensuring no radiative sunlight) in random locations within each population (60). This sampling allowed us to measure accurately the microclimate conditions experienced by lizards and classify population thermal conditions. Each year during almost a month (June 25 to July 21), we averaged the minimal daily temperature (T_{\min} , overnight temperature), the maximal daily temperature (T_{\max} , afternoon temperature peaks), and the global daily average (T_{mean}).

Sampling Design. We analyzed the impacts of life history, environmental conditions, and demographic trends on adult female and offspring TL in a subsample of nine sites, including two declining populations, in June 2017. Pregnant females ($n = 16$ per population) were measured, weighed, and acclimated in individual terraria following previously published procedures (60). In particular, they were provided with food (*Tenebrio molitor*) and water ad libitum until parturition. At arrival, we split pregnant females from each population randomly into two basking treatments to mimic different climate scenarios and associated changes in basking opportunities. A “cool” group of females had access for 3 h in the morning (9:00 to 12:00) to heat under an incandescent lightbulb (40 W) while the “warm” group was exposed for 9 h during the daytime (9:00 to 12:00 and 13:00 to 19:00). Note that a reference group (basking opportunity for 6 h in 8 additional females per population) was used as a control to extract parturition dates among years. We used HOBO temperature data loggers adapted with copper cast models to quantify differences in operative temperatures in randomly chosen terraria, which predicts the equilibrium body temperature of lizards in the absence of behavioral thermoregulation (61) (*SI Appendix, Fig. S4*). Females were checked twice a day to record their parturition date. After parturition, females were weighed and separated from their litter, given the absence of postbirth parental care. We counted and weighed all litter units, which included both successful (alive) offspring, stillborn or aborted embryos, and undeveloped eggs. Whenever possible, we determined offspring sex from the discriminant function linking the number of ventral scales and phenotypic sex with a success rate in determination above 96% (62). Females and litters were released at their capture sites within 2 d following birth.

Sample Collection, DNA Extraction, and TL Measurement. We sampled two different types of tissues in adult females and in their offspring. In adult

females, we collected red blood cells (RBCs) the day of capture using a faintly invasive protocol for blood sampling (~40 μL whole blood) from the postorbital sinus (63). RBCs were separated from plasma in a centrifuge at 11,000 rpm and immediately stored at -28°C until DNA extraction and molecular assays. In offspring, we collected tail tip tissues the day of birth, since their small body size (~21 mm SVL) makes blood samples too invasive and practically impossible in this age class. Tails are composed of a mix of skin, muscles, and bones and represent global individual TL, given that the tail is formed a few days after ovulation (64). Tail extremities (<2 to 3 mm) were cut with sterilized scissors and stored in a mix of pure ethanol (70%) and Tris-EDTA (buffer (30%)) at -28°C until molecular assays. Note that this species uses autotomy as a predation avoidance strategy and the tail will naturally regrow following autotomy in juveniles.

Molecular assays were performed by operators blinded to the sample origin (population, basking treatment, etc). DNA was extracted and purified using DNeasy blood and tissue kits (Qiagen) following manufacturer DNeasy 96 protocols for blood samples (adult females) or tissues samples (offspring). We assessed DNA concentration, quality, and integrity by optical density spectrophotometry (NanoDrop) and absorbance profile. The great majority of samples met high-quality standards (females: concentration = $62.8 \pm 23.7 \text{ ng}\cdot\mu\text{L}^{-1}$, absorbance ratio A260/280 = 1.94 ± 0.09 ; offspring: concentration = $56.1 \pm 33.2 \text{ ng}\cdot\mu\text{L}^{-1}$, absorbance ratio A260/280 = 1.89 ± 0.13). We then determined TL from 7.5 ng DNA using a real-time qPCR following a protocol previously developed and validated using tissue samples of this species (65). Briefly, telomeric DNA repeat sequences (T) were amplified using Tel1b and Tel2b primers and a reference, single-copy (S) gene (recombination activating gene 1, RAG-1) was amplified using primers designed with the *Z. vivipara* reference genome RAG-1 sequence. Samples were randomly distributed on nine plates and amplified in a qPCR thermocycler (Bio-Rad CFX96). We calculated TL as the T/S ratio of cycle thresholds following the procedure in ref. 66. Overall, amplifications showed high levels of efficiencies for both RAG-1 ($94.4 \pm 2.03\%$) and telomeric sequence ($89.8 \pm 2.81\%$). We further checked that a reference standard was repeatable between plates (T/S ratio ICC = 0.92) and that individual melting curves ensured unique amplifications.

Data Analyses. We ran all statistical analyses in the R software environment (67). We first built a PCA to characterize how breeding phenology (ordinal parturition date including latest and earliest dates of each population), probability of being recruited for yearlings (frequency of breeding yearlings in each population), and the average SVL of yearlings in each population covaried with thermal conditions (T_{min} , T_{max} , and T_{mean} of the study year), altitude of the population, and demographic trends (abundance changes from 2005 to 2017–2018) among the 22 sampled populations from the 2 study years. Given that basking treatments impacted parturition dates, we used females from control conditions (6 h) to focus solely on population differences. This PCA was built using the *ade4* library with centered and scaled variables (68). This procedure allowed us to rank populations along a composite score (PC1) describing concurrent changes in life history, climate conditions, and demographic changes across populations (SI Appendix, Fig. S1). Qualitatively similar results were obtained when we ranked the populations using solely the demographic trends to assess extinction risks (SI Appendix, Fig. S3). We next performed regression models with breakpoints using the *segmented* library (69) to update a linear mixed regression model linking TL data at a given age class with PC1 score of the population. All models included population identity as a random intercept term to account for nonindependence among observations. TL data were approximately normally distributed in offspring and adults and were z transformed for these analyses. Henceforth, population state was treated as a categorical effect (stable or declining) to optimize model convergence and ease interpretations of results.

In offspring, we used linear mixed models of the *nlme* library (70) to check whether TL variation was further explained by juvenile body size, date of birth, sex, basking conditions during gestation (hot or cool developmental conditions),

population state, mother TL (scaled by population state), and all first order interactions. All models accounted for additive random intercept of population identity and mother identity (sibling nonindependence). Model performance was averaged using the *dredge* function of the *MuMIn* library (SI Appendix, Table S2) (71). We also built a random model to obtain variance between family (σ^2_{family}), between population ($\sigma^2_{\text{population}}$), and within family ($\sigma^2_{\text{residuals}}$) to estimate broad-sense heritability in TL: $H^2 = 2 * \sigma^2_{\text{family}} / (\sigma^2_{\text{family}} + \sigma^2_{\text{population}} + \sigma^2_{\text{residuals}})$ (72).

In pregnant females, we examined the effect of population state, TL (scaled by population state), and first order interactions on reproductive performance using a generalized linear mixed model. We considered the total fecundity (the sum of all litter units) to quantify reproductive investment, and analyzed variation in total fecundity with Gaussian family models and maximum likelihood. We considered the number of alive offspring against the number of unsuccessful ones (stillborn aborted, undeveloped) as a marker of reproductive success and analyzed this response variable with a logistic regression, logit link, and binomial error term.

Data Availability. Data and codes used in this paper are available in SI Appendix and at Zenodo repository (DOI: 10.5281/zenodo.5798028).

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