Telomere length mirrors age structure along a 2200-m altitudinal gradient in a Mediterranean lizard

Pablo Burraco1,2,b,c,⁎, Mar Comas1,d,e, Senda Reguerad, Francisco Javier Zamora-Camachof, Gregorio Moreno-Ruedad

1 Animal Ecology, Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE-75236 Uppsala, Sweden
2 Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, United Kingdom
3 Ecology, Evolution and Development Group, Doñana Biological Station (CSIC), Spain
4 Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, Spain
5 Estación Biológica de Doñana (EBD-CSIC), Avda. Américo Vespucio 26, Seville E-41092, Spain
6 Departamento de Biogeografía y Cambio Global, Museo Nacional de Ciencias Naturales (CSIC), Spain

ARTICLE INFO

Keywords:
Altitude
Ageing
Body condition
Ectotherms
Life-history traits
Reptiles

ABSTRACT

The timing of organisms’ senescence is developmentally programmed but also shaped by the interaction between environmental inputs and life-history traits. In ectotherms, ageing dynamics are still poorly understood even though their body temperature, metabolism, or growth trajectory are very sensitive to environmental changes. Here, we investigated the role of life-history traits such as age, sex, body size, body condition, and tail autotomy (i.e. self-amputation) in shaping telomere length in six populations of the Algerian sand lizard (Psammodromus algirus) distributed along an elevational gradient from 300 to 2500 m above the sea level. Additionally, we compiled the available information on reptiles’ telomere length in a review table. Our cross-sectional study shows that older lizards have longer telomeres, which might be mostly linked to the selective disappearance of individuals with shorter telomeres or, alternatively, mediated by a higher expression of telomerase across their life. In fact, variation in telomere length across elevation was explained by age structure of lizards; thus, in contrast to our predictions, altitude had no effect on telomere length in this study system. Telomere length was unaffected by tail regeneration and was sex-independent, but positively correlated with body condition, which might be linked to high somatic investment. Hence, our results suggest that life-history traits such as age or body condition can be major drivers of telomere dynamics for this species, whereas environmental conditions apparently had scarce or no effects on lizard telomeres. Our findings emphasize the relevance of understanding species’ life histories for fully disentangling the causes and consequences of differences in ageing in ectotherms.

1. Introduction

Environmental conditions can modulate the physiology of individuals and therefore alter their ageing rate (Marasco et al., 2017; Ratikainen and Kokko, 2019). The study of the evolutionary underpinnings of ageing has been a long-standing topic both in ecological and medical research. Although several studies have shown the relation between mitochondrial metabolism and the variation in lifespan across taxa (Selman et al., 2012; Ziegler et al., 2015), the machinery governing ageing remains unclear. Most studies on vertebrate ageing have been conducted in endotherms. In contrast, ectothermic vertebrates have received less attention although their body temperature, metabolism, or growth trajectory are very sensitive to environmental changes, which might alter their ageing rate (Bronikowski, 2008; Olsson et al., 2018; Monaghan et al., 2018). Understanding the link between environmental conditions, life-histories, and senescence in wild ectotherms will increase the current knowledge about their evolutionary and ecological dynamics.

Telomeres are non-coding repeated sequences (TTAGGGn in vertebrates) located at the termini of chromosomes, essential for maintaining genomic stability and for protecting cells from chromosome degradation and fusion (O’Sullivan and Karlseder, 2010). Telomeres often shorten after each cell division, which likely explains that these regions tend to be shorter with age in endotherms, as observed in several mammals (e.g. Whittemore et al., 2019) and birds (e.g. Hall et al., 2004; Heidinger et al., 2012). However, the expression of the enzyme
| Reference               | Measured traits                                                                 | Relationship between TL and other traits | Relationship between TL and age | Population  | Technique | Tissue | Monitoring (sample size) | Common name | Species                  |
|------------------------|---------------------------------------------------------------------------------|------------------------------------------|---------------------------------|-------------|-----------|--------|---------------------------------|--------------|--------------------------|
| Girondot & Garcia (1998)| Age                                                                             | n.s. (embryos vs. adults)               |                                 | Wild        | TRF       | Blood  | Cross-sectional (N = 29)       | Emys orbicularis | European freshwater turtle |
| Scott et al. (2006)     | Body size, sex                                                                   |                                          | Body length (−), sex (m)        | Wild        | TRF       | Blood  | Cross-sectional (N = 26)       | Alligator mississippiensis | American alligator       |
| Bronikowski (2008)      | Age                                                                             | (−) (1-13 y/o)                          |                                 | Wild/lab    | TRF       | Blood  | Cross-sectional (N = 19)       | Thamnophis elegans   | Western terrestrial garter snake |
| Hatase et al. (2008)    | Age                                                                             | n.s. (0-36 y/o)                         |                                 | Captivity   | qPCR      | Blood, epidemis | Cross-sectional (N = 20) | Careta caretta | Loggerhead sea turtle |
| Ujvari and Madsen (2009)| Age, survival                                                                    | (+) for both sexes if including hatchlings (0-20 y/o), the same in longitudinal study (N = 8) |                                 | Wild        | TRF       | Blood  | Cross-sectional (N = 70)       | Liasis fuscus   | Water python              |
| Xu et al. (2009)        | Age, sex                                                                         | Foraging behaviour (−) for both sexes (3-10 y/o) |                                 | Captivity   | qPCR      | Blood   | Both (N = 30)                   | Chinese alligator   | Alligator sinensis        |
| Hatase et al. (2010)    | Foraging behaviour                                                               | Foraging behaviour (ns)                 |                                 | Wild        | qPCR      | Epidermis | Cross-sectional (N = 102)      | Loggerhead turtle   | Careta caretta          |
| Olsson et al. (2010)    | Length, age, activity, ticks, tail regeneration (−) for females, (−) trend for males (2-8 y/o) | Females: length (ns), activity (ns), ticks (ns), tail regeneration (m), males: ticks (ns), badge size (ns), activity (−), length (−), tail regeneration (−) | Wild        | TRF       | Blood  | Cross-sectional (N = 124)      | Sand lizard        | Lacerta agilis           |
| Olsson et al. (2011a)   | Heritability, paternal age, offspring survival, malformations (−) for sires, n.s. for sons (3-7 y/o) | Capture probability of sires (+), offspring sex-ratio (−) TL of sons and paternal age at conception (−) | Wild        | TRF       | Blood  | Cross-sectional (N = 93)       | Sand lizard        | Lacerta agilis           |
| Olsson et al. (2011b)   | Sex                                                                             | Longer TL in females than in males; females: lifespan (+), lifetime reproductive success (+), males: lifespan (ns), lifetime reproductive success (ns) | Wild        | TRF       | Blood  | Cross-sectional (N = 190)      | Sand lizard        | Lacerta agilis           |
| Ballen et al. (2012)    | Maternal and offspring TL, body mass, superoxide (−) for sires, n.s. for some (3-7 y/o) | Offspring TL with maternal TL (−), maternal reproductive investment (−), offspring mass (−), offspring superoxide (−) | Wild/lab    | PNA Kit/FITC flow cytometry | Blood  | Cross-sectional (N = 14)       | Painted dragon   | Ctenophorus pictus       |
| Plot et al. (2012)      | Sex, age, reproduction                                                           | Reproductive output (−), time to first breeding (−) | Wild        | qPCR      | Blood  | Cross-sectional (N = 42)       | Leatherback sea turtle | Dermochelys coriacea |
| Giraudoue et al. (2016) | Colour fading                                                                   | Colour fading (−)                        | Wild        | qPCR      | Blood  | Longitudinal (N = 53)          | Painted dragon    | Ctenophorus pictus       |
| Rollings et al. (2017a) | Head colour, bib presence                                                        | Competition ability (−), bib presence (−) | Wild/lab    | qPCR      | Blood  | Cross-sectional (N = 73)       | Painted dragon    | Ctenophorus pictus       |
| Dupoué et al. (2017)    | Body size, sex, altitude, extinction risk, Tmin (−), Tmx (−)                    | Body size (ms), sex (ns), extinction risk (−), altitude (−), Tmin (−) | Wild        | TRF       | Blood  | Cross-sectional (N = 100)      | Common lizard     | Zootoca vivipara         |
| Rollings et al. (2017b) | Age, sex                                                                         | Shorter TL in males, body length (ns), growth (ms), body condition (−) | Wild        | qPCR      | Blood  | Cross-sectional (N = 150)      | Red-sided garter snake | Thamnophis sirtalis parietalis |
| Ujvari et al. (2017)    | Age, survival                                                                    | Survival (ms)                           | Wild        | qPCR      | Blood  | Both (N = 93)                   | Frillneck lizard  | Chlamydosaurus kingii     |
| Pauliny et al. (2018)   | Paternity probability                                                            | Probability of siring offspring (−)      | Wild/Lab    | qPCR      | Blood  | Cross-sectional (N = 80)       | Sand lizard        | Lacerta agilis           |
| Zhang et al. (2018)     | Temperature                                                                      | Temperature (−)                          | Laboratory | qPCR      | Heart   | Cross-sectional (N = 80)       | Desert toad-headed agama | Phrynocephalus prasinusbai |
| Rollings et al. (2019)  | Sex, tissue                                                                      | TL varies between sexes and among tissues | Wild        | qPCR      | Several | Cross-sectional (N = 24)       | Painted dragon    | Ctenophorus pictus       |
| Mckenzie et al. (2019)  | Reproductive mode                                                                | Longer TL in viviparous mothers and offspring than in oviparous | Wild/lab    | qPCR      | Tail   | Cross-sectional (N = 126)      | Common lizard     | Zootoca vivipara         |
| Singchet et al. (2019)  | Age, sex                                                                         | (−) for males, quadratic for females (0-11 y/o) | Wild        | qPCR      | Blood  | Cross-sectional (N = 80)       | Siamese cobra     | Naja kaouthia              |

(continued on next page)
Table 1 (continued)

| Species                        | Common name                        | Tissue         | Monitoring (sample size) | Population | Technique | Tissue Monitoring |
|--------------------------------|------------------------------------|----------------|--------------------------|------------|-----------|------------------|
| *Niveoscincus ocellatus*        | Ocellated cool-skink                | Blood          | Laboratory qPCR          | Captivity  | Wild      | Laboratory       |
| *Lacerta agilis*                | Sand lizard                         | Blood          | Laboratory qPCR          | Captivity  | Wild      | Laboratory       |
| *Zootoca vivipara*              | Common lizard                       | Blood          | Laboratory qPCR          | Captivity  | Captivity | Laboratory       |

References are included in the Supplementary Material.

3. Telomerase can alleviate telomere erosion. Telomerase is known to be active across development in some ectothermic vertebrates (Knappert et al., 1998; Bousman et al., 2003). In this line, signs of telomere elongation have been found throughout larval development of the Atlantic salmon (*Salmo salar*, McLennan et al., 2016) and of the common water frog (*Rana temporaria*; Burraco et al., 2019), as well as during the first years of life in some reptiles (e.g. Olsson et al., 2010; Ujvari et al., 2017). Telomere length, at a given ontogenetic point, is not only a function of cell replication but also of the organisms’ ability to cope with stress across their life (Dugdale and Richardson, 2018). In vertebrates, harmful conditions often enhance glucocorticoids secretion, which can induce an oxidative state and damage essential biomolecules like lipids, proteins or DNA (Luceri et al., 2018; Florencio et al., 2020), including telomeres (Reichert and Stier, 2017; but see Chatelain et al., 2020). As a consequence of the apparent sensitivity of telomeric sequences to environmental inputs, telomere length is often used as an indicator of the amount of stress accumulated by an organism across time (Young, 2018). Several studies across taxa have found positive relationships between telomere length and organisms’ life expectancy (e.g. Heidinger et al., 2012; Barrett et al., 2013; Wilbourn et al., 2018), reproductive outcome (Eastwood et al., 2019), or immunocompetence (Alder et al., 2018). In ectothermic vertebrates, telomere shortening has been associated with increased growth rate, bold personality, or predator exposure (reviewed in Olsson et al., 2018).

In reptiles, a paraphyletic group, several studies have investigated the variation in telomere length with age (Table 1). Five studies found that telomeres shorten with age, although in some cases this relationship was sex-dependent (Table 1). A quadratic sex-dependent relationship between telomere length and age was observed in three studies, i.e. telomeres increase their length until a certain age, and then shorten (Table 1). Meanwhile, three studies found no effect of age on telomere length (Table 1). The high inter-species variation in the relation between telomere length and age highlights the need of further research. Furthermore, several studies have addressed the relationship between telomere length and some life-history traits. For instances, some studies show a positive relationship between telomere length and lifetime reproductive success or body condition. In contrast, no association or unexpected relationships were observed for other traits, or it was species-dependent (Table 1). For instance, one might expect telomeres to shorten as body size increases across lifetime, since it implies a higher number or rate of cellular replications. However, only a few studies on reptiles have observed a significant negative effect of body size or growth rate on telomere length (Table 1), unlike in fish (McLennan et al., 2016) or amphibians (Burraco et al., 2017a). Therefore, so far, we should not generalise when discussing telomere dynamics in reptiles, further studies being needed.

Here, we also aim to understand the role of elevation on telomere length of the Algerian sand lizard (*Psammodromus algirus*). To this end, we studied a substantial altitudinal gradient from 300 to 2500 above sea level (m.a.s.l. thereafter) in Sierra Nevada mountain system (Spain). Mounts cover a quarter of the Earth’s surface and show deep variations across the elevational gradient in biotic and physical conditions, such as predator abundance, temperature, or ultraviolet radiation (Körner et al., 2011). Consequently, physiological adaptations to divergent habitats across altitude have been reported in different taxa (Keller et al., 2013). Such physiological adjustments often imply elevational variation in the relative allocation of energy expenditure to reproduction and somatic maintenance (Bronikowski and Arnold, 1999), which can affect telomere dynamics (Stier et al., 2016). In this altitudinal gradient, as a consequence of environmental temperature decrease with altitude, these lizards reduce their activity while hibernation time increases with ascending elevation (Zamora-Camacho et al., 2013). Hibernation is known to slow down telomere attrition (Hoelzl et al., 2016; Kirby et al., 2019), hence telomere dynamics of lizards at higher altitudes might benefit from longer hibernation. On the other hand, the Algerian sand lizard is heterothermic, meaning that they...
spend a notable amount of time exposed to solar radiation. This lizard devotes more time to basking with increasing elevation (Díaz, 1997), where UV radiation is higher (Reguera et al., 2014a), thereby compensating the dwindling environmental temperature (Zamora-Camacho et al., 2013; Zamora-Camacho et al., 2016). The exposure to UV radiation is known to damage DNA (Cadet et al., 2015), which might negatively affect telomeres in high-elevation lizards. However, lizards at high elevations are darker, which may protect them from UV radiation (Reguera et al., 2014a). Probably as a consequence of reduced activity time, elongated hibernation, and darker colorations, oxidative stress is lower in high-elevation lizards (Reguera et al., 2014b; Reguera et al., 2015). Overall, according to the information gathered on this species along the elevational gradient, we predicted longer telomeres at high elevations.

The main goal of our study was to investigate telomere length across altitude in a lizard species. However, other factors may potentially affect telomere dynamics. *P. algirus* lizards do not show a noticeable sexual dimorphism, as both sexes often have similar body sizes, although males can show orange or blue colorations (Carretero, 2002). Therefore, we did not expect differences in telomere length between sexes beyond the putative costs linked to reproductive investment linked to each sex. Importantly, and given that telomere length is often affected by age, we estimated the age of lizards. Regarding the available information on the relationship between telomere length and age in reptiles, one might expect either a positive, negative, or quadratic relationship between both factors (Table 1). Overall, we aimed to increase the available information on telomere length in reptiles, thus helping to gradually fill the gap of knowledge on telomeres in ectothermic vertebrates.

2. Material and methods

2.1. General procedures

The lizard *P. algirus* is a medium-large lacertid (53–80 mm snout-vent length –SVL- in our study area) that inhabits shrubby habitats in the Mediterranean region from south-western Europe and north-western Africa (Salvador, 2015). In the Sierra Nevada mountain system (SE Spain), we sampled individuals from six populations, at 300 (N = 18), 700 (N = 16), 1200 (N = 18), 1700 (N = 19), 2200 (N = 15), and 2500 (N = 20) m.a.s.l. (Fig. 1). In total, we assessed 106 individuals (50 males and 56 females): 7 in 2010, 28 in 2011, 65 in 2012 and 6 in 2013. Additionally, we estimated telomere length in 37 neonates (see below). Because lizards were part of a long-term study, we marked individuals by toe clipping, a marking method frequently used in lizards, with limited impact on their welfare (Perry et al., 2011). We conserved toe samples in ethanol and used them for age class determination using phalanx skeletochronology (more details below). We collected a portion of the terminal region of lizards’ tail (~ 1 cm) in the wild and stored tail samples at −20 °C until analysis. We used a high-salt DNA extraction protocol (Lahiri and Nurnberger Jr., 1991). This method eliminates the use of toxic reagents such as phenol or chloroform, and yields high amount of good-quality DNA. We used a Nanodrop (Thermo Scientific) to quantify DNA concentration and quality. Since storage conditions, extraction method, or tissue type can affect telomere length measurements (Nussey et al., 2014) we used the same conditions for all samples to avoid confounding factors.

Telomere length was measured using a golden standard that includes the cycle threshold (Ct) of telomeric sequences with the Ct of a control sequence that is autosomal and non-variable in copy number (Cawthon, 2002; Nussey et al., 2014). As a reference sequence, we amplified GAPDH sequences using 5′-AACCAGCAAGTACGATGACAT-3′ (GAPDH-F) and 5′-CCATCAGCA GCAGCTCTCCA-3′ (GAPDH-R) as forward and reverse primers, respectively. The use of GAPDH as a single copy gene is widely spread in telomere studies in vertebrates and has been previously used in sand lizards (Pauliny et al., 2018). We confirmed that the amplification efficiency was low for this gene (the average Cq-value was 25.37 with a standard error (S.E.) of ±0.32). For telomere sequences, we used 5′-CGGTGTTTGGGTGGTGTTTGGTGTTGTTGTTGTTGTT-3′ (Tel2b) and 5′-GGCTTGGCTTACCTACCTTACCTACCTTACCTTACCTTACCTTACCT-3′ (Tel2b) as forward and reverse primers, respectively. Conditions of qPCR for GAPDH fragment consisted of 10 min at 95 °C and 40 cycles of 10 s at 95 °C, 20 s at 58 °C, and 1 min at 72 °C, and for telomere fragment of 10 min at 95 °C, and 10 s at 95 °C, 20 s at 58 °C, and 1 min at 72 °C. We conducted qPCR assays for each gene in separate plates on a LightCycler 480 (Roche) and ran a melting curve from 65 to 95 °C, as a final step in each qPCR to check for specific amplicons. Melting curve showed a normal shape, indicating the high specificity of GAPDH and telomere primers (Supplementary Material S1). For each sample, we added 20 ng of genomic DNA and used both sets of primers at a final concentration of 100 nM in a 20 μL master mix containing 10 μL of Brilliant SYBR Green (QPCR Master Mix, Roche). All samples were run in duplicate. Samples with coefficient of variation higher than 5% were measured again. We calculated qPCR-plates efficiency by including five serial diluted standards in triplicate (120, 40, 10, 2.5 and 0.66 ng/μL, for GAPDH and telomere sequences), obtained from a golden standard sample containing a pool of three samples from each elevation. We calculated relative telomere length by applying the following formula (Pfaffl, 2001): \[ \frac{\Delta Ct_{telomere} - \Delta Ct_{GAPDH}}{\Delta Ct_{GAPDH}} \times 100 \] where \( \Delta Ct_{telomere} \) and \( \Delta Ct_{GAPDH} \) are the qPCR efficiency of telomere and GAPDH fragment, respectively; \( \Delta Ct \) telomere (control-sample) and \( \Delta Ct \) GAPDH (control-sample) are the deviation of standard – telomere or GAPDH sequences, respectively. The inr-assay CV% was 4.07% for GAPDH gene and 1.38% for telomere gene. The inter-assay
CV% was 11.26% for relative telomere length. All the R² of the standard curves were higher than 0.985.

2.3. Estimation of age class with skeletochronology

We estimated individual age class by phalanx skeletochronology (Comas et al., 2019), one of the most accurate techniques to estimate age in many vertebrates, including reptiles (Zhao et al., 2019). Vertebrate ectotherms show indeterminate growth, and consequently present a cyclic growth pattern in hard body structures such as bones, corresponding to alternate periods of growth and resting. This pattern is particularly marked in temperate climates, where age can be fairly estimated by counting annual growth rings in the phalanges (Comas et al., 2016). Growth rings are called lines of arrested growth (LAGs). Toes sampled were decalcified in 3% nitric acid for 3 h and 30 min. Cross-sections (10 μm) were prepared using a freezing microtome (CM1850 Leica), stained with Harris hematoxylin for 20 min and dehydrated through an alcohol chain (more details in Comas et al., 2016). Next, cross-sections were fixed with DPX (mounting medium for histology), mounted on slides, and examined for the presence of LAGs using a light microscope (Leitz Dialux20) at magnifications from 50 to 125×. We took several photographs (with a ProgresC3 camera, at the University of Barcelona UB) of various representative cross-sections, discarding those photographs in which cuts were unsuitable for observing LAGs.

The number of LAGs detected in the periosteal bone was independently and blindly counted three times by a single observer (MC) on three independent occasions.

2.4. Statistical analysis

In order to meet parametric assumptions, we log-transformed relative telomere length, body mass, and body condition data. We examined the presence of outliers through a Cleveland plot, which revealed that an individual had an extremely abnormal low value (almost zero) of relative telomere length, so we decided to omit this datum from all the analyses. Cleveland plots also showed a possible outlier within the body condition data, thus we followed the recommendations of Quinn and Keough (2002) and, for analyses implying body condition data, we performed the test with and without the datum and reported both statistical results.

Given that not all individuals had associated data for all variables (e.g., neonates always had complete tails and were not sexed) and some variables presented collinearity (e.g. SVL and age), we first ran some analyses in order to test whether variables potentially affecting telomere dynamics covaried with either relative telomere length or altitude. We performed linear models to check for sexual differences in body mass, age or relative telomere length. A chi-squared test was used to test whether the frequency of males and females differed with the number of LAGs detected in the periosteal bone.
elevation. A linear model was also used to test relative telomere length according to the year of capture in order to evaluate possible cohort effects. Since we sampled lizards with intact tails \((n = 44)\) and regenerated tails \((n = 58)\), and tail regeneration could affect telomere dynamics in tail tissue (Alibardi, 2016), we tested whether there were differences in relative telomere length between lizards with intact or regenerated tail through linear models. A chi-squared test was used to test whether the frequency of individuals with intact or regenerated tails differed with elevation.

We also performed linear models to test how relative telomere length varied with age class. In these models, given that there were only five lizards 4 years old and two lizards 5 years old, age was reclassified as neonate, 1, 2, and ≥ 3 years. Moreover, we repeated the analysis without including lizards with 4 and 5 years old. We used Pearson correlations to test for the covariation between relative telomere length and variables such as age, body mass, SVL, and body condition, and for the relationships between age and both body mass or SVL. Using a linear model, we tested for the variation in body condition with altitude.

Finally, we tested the altitudinal variation in relative telomere length in lizards. Given that both altitude and age class were significantly related to relative telomere length, we tested the effect of the two variables as predictors on relative telomere length, as a dependent variable. We also tested for the altitudinal variation in telomere length for neonates, in order to check for the variation in telomere length with altitude at birth. For all the linear models, we confirmed that data met parametric assumptions. All statistical analyses were conducted in Statistica software (version 8.0).

3. Results

Lizards did not show sexual dimorphism in body mass (\(F_{1, 102} = 0.11, P = .74\)) and average age was similar for both sexes \((F_{1, 103} = 1.70, P = .20)\). The frequency of male and female lizards did not differ across lizard populations \((\chi^2 = 0.66, P = .98)\). Relative telomere length did not differ between sexes \((F_{1, 103} = 0.30, P = .59; \text{Fig. 2A}; \text{Supplementary Material S2})\). The year of capture did not affect telomere length either \((F_{3, 101} = 0.45, P = .72)\). The frequency of lizards with regenerated tails did not vary among lizard populations \((\chi^2 = 1.36, P = .93)\), and tail regeneration did not affect lizard telomere length \((F_{1, 100} < 0.01, P = .99; \text{Fig. 2B}; \text{Supplementary Material S2})\).

Individuals in the different age classes had different mean relative telomere length \((F_{3, 138} = 3.41, P = .019, \text{Fig. 3}; \text{Supplementary Material S2})\).
When individuals with four and 5 years are not included in the model, the finding is marginally non-significant ($F_{3, 131} = 2.42, P = .069$). Pearson correlations between telomere length and age showed similar results ($r = 0.25, P = .002$, including neonates, $N = 147$; $r = 0.19, P = .047$, without neonates, $N = 105$; $r = 0.22, P = .010$, without individuals older than 3 years, $N = 135$). Age correlated positively with body mass ($r = 0.57, P < .001$) and SVL ($r = 0.59, P < .001$; Supplementary Material S2), which is common in organisms with indeterminate growth like lizards. Likewise, larger individuals had longer telomeres (with SVL, $r = 0.21, P = .036$; with body mass, $r = 0.26, P = .007$; Fig. 4). Relative telomere length tended to increase with body condition ($r = 0.18, P = .067$). This relationship became significant ($r = 0.20, P = .043$) when a possible outlier—an individual with very high body condition—was removed (Fig. 5A). Body condition increased with elevation ($F_{5, 98} = 3.03, P = .014$; Fig. 5B).

Lizard telomere length differed among populations across this elevational gradient following a non-linear pattern ($F_{5,136} = 2.52; P = .03$ for all individuals, and $F_{5,99} = 2.07; P = .070$ when excluding neonates; Fig. 6; Supplementary Material S2). However, average age varied with elevation in a similar way ($F_{5,131} = 5.44; P < .001$; Supplementary Material S3). When we tested for the combined effect of age and elevation on telomere length, the effect of age remained significant ($F_{5,131} = 2.32; P = .047$), but the effect of elevation was no longer significant ($F_{5,131} = 1.67; P = .15$). Neonate telomere length, an indicator of the baseline telomere length at birth, varied among populations ($F_{5,31} = 2.91; P = .03$), but with no clear pattern; lizard neonates showed the longest telomeres at 2200 m.a.s.l., but the shortest at 1700 m.a.s.l. (Supplementary Material S4).

4. Discussion

Life-history trade-offs and environmental conditions can shape ageing across taxa (Wilbourn et al., 2018; Eastwood et al., 2019). In our study system, we expected to find Algerian sand lizards with longer telomeres at high elevation. This hypothesis was based on the grounds that ectotherms typically live longer at high altitude (Cabezas-Cartes et al., 2018) and that the populations studied herein show reduced time of activity and oxidative stress with elevation (see Introduction, Zamora-Camacho et al., 2013; Reguera et al., 2014b). However, our hypothesis was not supported by the data, as lizards showed an altitudinal pattern of telomere length that simply mirrored the altitudinal distribution in average age. Our cross-sectional study also suggests that, in these lizards, telomeres are longer with age, although we acknowledge the putative role of selective disappearance in explaining this pattern, as discussed below. Likewise, older (and thus larger) lizards...
had longer telomeres. Moreover, our findings suggest that differences in telomere length were sex-independent, unlike adult sand lizards of other species (Lacerta agilis, Olsson et al., 2011). Sex differences in telomere length may result from sex differences in growth rate, body size, and/or age (Olsson et al., 2018). However, in our study system, lizards did not show sexual dimorphism in size or age structure. Tail regeneration did not affect telomere length despite the fact that differences in the regulation of telomere length may be driven by evolutionary pressures such as predation (Olsson et al., 2010), and also by metabolic demands during tissue regeneration. Moreover, no cohort effect was detected, as telomere length did not differ with year of sampling.

Longer telomeres were associated with older age in lizards. This result agrees with previous studies in snakes and lizards (Ujvari and Madsen, 2009 and Ujvari et al., 2017, respectively). We also found a positive relationship between telomere length and body size. Although telomere length and survival had no association in other lizards such as in the frillneck lizard (Chlamydosaurus kingii, Ujvari et al., 2017), larger body size can include lower mortality risk in ectotherms with indeterminate growth (Angilletta Jr et al., 2004). If extrinsic conditions selectively remove individuals in poor condition—with expected shorter telomeres and likely smaller body size—, then the fact that older lizards have longer telomeres might indicate a prolonged survival of individuals in better condition (Van de Pol and Wright, 2009; Salmón et al., 2017). Despite the putative role of selective disappearance in explaining differences in telomere length, the positive relationship between telomere length and body size suggests that increase in body size—likely involving higher cell replication—does not imply shorter telomeres by itself. Previous studies have shown that ectotherms, unlike endotherms, can show longer telomeres along their lifetime (Olsson et al., 2018). Such contrasting patterns of telomere dynamics may be related to a higher telomerase expression after birth in somatic cells in ectotherms than in endotherms (Gomes et al., 2010). Hence, telomerase may be relevant for buffering downstream effects of cellular damage in organisms with indeterminate growth such as lizards (Jones et al., 2014). However, telomerase expression may not be enough to protect from telomere shortening in ectothermic vertebrates. For instance, telomerase is expressed in tissues of adult medaka fish (Klapper et al., 1998) but telomeres shorten with age in this species (Hatakeyama et al., 2008). Furthermore, the maintenance of telomerase expression in species with indeterminate growth can imply a trade-off suggested by a higher cancer occurrence in ectotherms (Gomes et al., 2010; Olsson et al., 2018). However, the knowledge about cancer in wildlife is still meagre. In our study system, the use of a longitudinal approach would allow to disentangle the possible role of selective disappearance or telomere elongation (and telomerase activity) in explaining differences in telomere length in older lizards.

We expected longer telomeres in populations at higher elevation. However, elevation did not shape telomeres in these lizard populations since the variation in telomere length was explained by the age structure at each altitude. Contrary to our results, Dupoué et al. (2017) found shorter telomeres and higher extinction risk in low-elevation populations of the common lizard (Zootoca vivipara). In our study system, lowland populations suffer poor habitat quality since they face low thermal quality (risk of overheating, Zamora-Camacho et al., 2016), high ectoparasitism (Álvarez-Ruiz et al., 2018), low food availability (Moreno-Rueda et al., 2017), and high oxidative damage (Reguera et al., 2014b, 2015). Additionally, low-elevations lizards increase their activity time while decreasing hibernation time (Zamora-Camacho et al., 2013). Nonetheless, low-elevation populations did not have shorter telomeres than populations at high elevations.

Lizard body condition, environmental temperature, oxidative stress, and telomerase expression might explain the lack of variation in telomere length in lizards inhabiting at different elevations. In this study, body condition of lizards was greater in populations at higher elevation and correlated positively with telomere length. It is known that telomere length can show a positive correlation with body condition in other reptiles (Thamnophis sirtalis; Rollings et al., 2017), suggesting that body condition is an indirect measure of somatic investment. In addition, a temperature-mediated regulation of telomerase expression is likely, thus telomerase might show a higher expression at low elevation, then compensating for telomere erosion (Olsson et al., 2018; Fitzpatrick et al., 2019). At high elevations, the reduction in metabolic rate due to cold conditions may have favoured a reduction in the rate of telomere erosion due to a reduced production of ROS (Reguera et al., 2014b, 2015). Indeed, increases in lifespan are often orchestrated by reductions in metabolic rate (Speakman, 2005), as for example show in the snake Thamnophis elegans across an elevational gradient (Bronikowski and Vleck, 2010). Furthermore, the variation in the pace-of-life as a consequence of particular environmental conditions is also known to alter telomeres, thus resulting in complex or unexpected patterns (Giraudneau et al., 2019). In ectotherms, shorter telomeres are associated with higher survival in migratory Atlantic salmon (McLennan et al., 2017), which may indicate a trade-off between investment in migration and investment in telomere maintenance. Likewise, amphibian larvae inhabiting ponds under permanent desiccation risk, showed shorter telomeres (Burraco et al., 2017b). In our system, other factors like diseases or intraspecific interactions might have also modulated ageing in lizards at each elevation. A cross-fostering approach would help to fully clarify the evolutionary impact of both environment and life-history traits on telomeres of this lizard metapopulation.

5. Conclusions

In contrast to our expectations, altitude had no effect on lizard telomere length. Our results suggest that telomeres are longer with age, and telomere length variation with elevation reflects variation in age along the mountain gradient. Nevertheless, in our cross-sectional study, we cannot disentangle whether this age-dependent variation in telomere length is due to telomere elongation with age or to selective disappearance of low-quality individuals with shorter telomeres.
Likewise, larger lizards (and those with higher body condition) had longer telomeres. This study highlights the relevance of understanding species’ life histories and habitat characteristics for disentangling the causes and consequences of differences in aging in ectotherms.

Data availability

Data are accessible at FigShare repository: https://figshare.com/articles/Dataset_of_Burraco_et_al_2020_in_CBPA/12452759.

Declaration of competing interest

No conflict of interest declared.

Acknowledgements

PB was supported by a fellowship F.P.U.-AP2010-5373, by the Carl Tryggars Foundation project CT 16:344, and by a Marie-Curie Fellowship METAGE979879, FIZC (F.P.U.-AP2009-3505) and SR (F.P.U.-AP2009-1325) also were supported by respective fellowships. MC was supported by a Severo Ochoa contract (SVP-2014-068620). This study was economically supported by the Ministerio de Ciencia e Innovación (project CGL2009-13185). The study complies with the current laws of Spain, and were performed in accordance with the Junta de Andalucía and Sierra Nevada National Park research permits (references GMN/Gyb/JMIF and ENSN/JSG/JEGT/MCF). We are grateful to Concepción Hernández (Centre of Scientific Instrumentation of the University of Granada) for her help with the freezing microtome, Humbert Salvador (Universitat of Barcelona) for let us to use his microscopy for this study, and Francisco Miranda (Ecophysiology Laboratory of Doñana Biological Station) for assistance with the telo- 

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2020.110741.

References

Alder, J.K., Hamanathan, V.S., Strong, M.A., DeZere, A.E., Stanley, S.E., Takemoto, C.M., ... Bruday, R.A., 2018. Diagnostic utility of telomere length testing in a hospital-based setting. Proceedings of the National Academy of Sciences 115 (10), E2288-E2365.

Allard, L., 2016. Immunocalcification of telomeres in cells of lizard tail after amputation suggests cell activation for tail regeneration. Tissue Cell 48, 63-71.

Álvarez-Ruiz, L., Megal-Palma, R., Reguera, S., Ruiz, S., Zamora-Camacho, F.J., Figueroa, J., Moreno-Rueda, G., 2018. Opposed elevational variation in prevalence and intensity of endoparasites and their vectors in a lizard. Curr. Zool. 64 (2), 197-204.

Angilleta Jr., M.J., Steury, T.D., Sears, M.W., 2004. Temperature, growth rate, and body length and dynamics predict mortality in a wild longitudinal study. Mol. Ecol. 22 (1), 2025-2043.

Kirby, R., Johnson, H.E., Allardige, M.W., Pauli, J.N., 2019. The cascading effects of temperature and telomere length: thermal treatment influences telomere dynamics through a complex interplay of cellular processes in a cold-climate skin. Oecologia 191 (1), 767-776.

Burraco, P., Díaz-Paniagua, C., Gomez-Mestre, I., 2017a. Different effects of accelerated growth rate and developmental rate on life history traits of an amphibian. Integr. Comp. Biol. 58 (17), 1743-1748.

Heidinger, B.J., Blount, J.D., Boner, W., Griffiths, K., Metcalfe, N.B., Monaghan, P., 2012. Telomere length in early life predicts lifespan. Proc. Natl. Acad. Sci. 109 (5), 2017-2048.

Higham, T.E., Russell, A.P., Zani, P.A., 2013. Integrative biology of tail autotomy in lizards. Physiol. Biochem. Zool. 86 (6), 603-610.

Hofeld, E., Cornisch, J.S., Smith, M., Moodley, Y., Ruf, T., 2016. Telomere dynamics in free-living edible dormice (Glis glis): the impact of hibernation and food supply. J. Exp. Biol. 219 (16), 2469-2474.

Jones, O.R., Schuelein, A., Salguiero-Gomez, R., Camara, C.G., Schable, R., Casper, R.B., Quintana-Ascencio, P.F., 2014. Diversity of ageing across the tree of life. Nature 505 (7482), 169.

Keller, I., Alexander, J.M., Holderegger, R., Edwards, P.D., 2013. Widespread phenotypic and genetic divergence along altitudinal gradients in animals. J. Evol. Biol. 26, 2527-2543.

Kirby, R., Johnson, H.E., Allardige, M.W., Pauli, J.N., 2019. The cascading effects of human food on hibernation and cellular aging in free-ranging black bears. Sci. Rep. 9 (1), 2197.

Klapper, W., Heidorn, K., Kühne, K., Parwesec, R., Krupp, G., 1998. Telomerase activity in ‘immortal’ fish. FEBS Lett. 434 (3), 409-412.

Körner, C., Paulsen, J., Spina, E.M., 2011. A definition of mountains and their bioclimatic belts for global comparisons of biodiversity data. Alp. Bot. 121 (2), 73-78.

Lahl, D.K., Nurnberger Jr., J., 1991. A rapid non-enzymatic method for the preparation of HMM DNA from blood for RFLP studies. Nucleic Acids Res. 19, 5444.

Luceri, C., Biggali, E., Femina, A.P., Caderni, G., Giovannelli, L., Lodovici, M., 2018. Aging related changes in circulating reactive oxygen species (ROS) and protein carbonyls are indicative of liver oxidative stress. Toxicol. Rep. 5, 141-145.

Marasco, V., Suter, A., Boner, W., Griffiths, K., Heidinger, B.J., Monaghan, P., 2017. Environmental conditions can modulate the links among oxidative stress, age, and longevity. Aging Cell. 16, 100-107.

McIvor, A.R., Armstrong, J.D., Stewart, D.C., McKelvey, S., Boner, W., Monaghan, P., Metcalfe, N.B., 2016. Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. Mol. Ecol. 25 (12), 5425-5438.

McIvor, A.R., Armstrong, J.D., Stewart, D.C., McKelvey, S., Boner, W., Monaghan, P., Metcalfe, N.B., 2017. Shorter juvenile telomere length is associated with higher survival to spawning in migratory Atlantic salmon. Funct. Ecol. 31 (11), 2070-2079.

Monaghan, P., Eisenberg, D.T., Harrington, L., Nussey, D., 2018. Understanding diversity
