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The association between age and telomere length is age-dependent: Evidence for a threshold model of telomere length maintenance

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Abstract

Telomere length and dynamics are commonly used biomarkers of somatic state, yet the role of telomeres underlying the aging process is still debated. Indeed, to date, empirical evidence for an association between age and telomere length is mixed. Here, we test if the age-dependency of the association between age and telomere length can provide a potential explanation for the reported inconsistencies across studies. To this end, we quantified telomere length by telomere restriction fragment analysis in two groups of Japanese quail (*Coturnix japonica*) that differed in their age distribution. One group consisted of young adults only, whereas the second group consisted of adults across a wide range of ages. In the young adults group, there was a highly significant negative association between telomere length and age, whereas no association between age and telomere length was found in the all-ages adults group. This difference between groups was not due to telomere length-dependent selective disappearance. Our results shows that the association between telomere length and age is age-dependent and suggest that the costs and benefits associated with telomere maintenance are dynamic across an individual's life course.

KEYWORDS

ageing, evolutionary theory of ageing, evolutionary trade-offs, life-history strategies, telomere shortening and attrition

1 | INTRODUCTION

Over the last two decades, the interest in telomere length and dynamics as biomarkers of individual somatic state has increased exponentially (Lopez-Otin et al., 2013; Tobler et al., 2022). Telomeres are highly repetitive noncoding DNA sequences at the end of eukaryotic chromosomes that protect the genetic material during cell division, and safeguard genomic integrity and cell viability (Blackburn, 1991). Telomeres shorten with each cell division (Harley et al., 1992), and by extrapolating from cellular to individual level, it is typically assumed that they shorten throughout an individual's life (Aviv et al., 2015; Tricola et al., 2018), resulting in a negative association between telomere length and age. Meta-analyses in humans (Muezzinler et al., 2013) and non-human vertebrates (Remot et al., 2021) found evidence for such a trend, however overall the effect is surprisingly weak and many studies failed to find evidence for a negative relationship (Remot et al., 2021; Tricola

et al., 2018). These inconsistencies may partly be explained by methodological differences in telomere length measurements (e.g., quantitative polymerase chain reaction vs. telomere restriction fragment [TRF], [Nussey et al., 2014; Remot et al., 2021]). However, they may also have biological explanations. In particular, patterns of telomere shortening and telomere maintenance may vary across the life course, leading to strong associations between age and telomere length at some life stages, but not at others (Angelier et al., 2018; Remot et al., 2021).

Indeed, the evolutionary theory of aging predicts that selection on self-maintenance will be stronger early in life than later in life, either because deterioration later in life is not (strongly) selected against (Medawar, 1952) or because alleles with late-life deleterious effects have early life benefits (Williams, 1957). Based on this theory, mechanisms that prevent or delay telomere shortening are predicted to act more strongly during early adulthood, leading to a stronger association between age and telomere length later in life (Figure 1). Alternatively, telomere length maintenance may follow a threshold model (Aviv et al., 2015; Hasselquist & Tobler, 2021; Tobler et al., 2022), which proposes that telomere shortening has no functional consequences—and is thus not prevented—until a critical telomere length (also called "telomeric brink," [Aviv et al., 2015]) is reached, but further shortening beyond this critical point is actively prevented. Based on this model, mechanisms that prevent telomere shortening are predicted to act more strongly during later life, leading to a stronger association between age and telomere length early in life (Figure 1). In line with this latter scenario, a particularly strong telomere attrition early in life has been observed in several species (e.g., Hall et al., 2004; Heidinger et al., 2012; Roast et al., 2022). Thus, depending on the age distribution of the study population, and the relative importance of the two scenarios outlined above, associations between age and telomere length may be absent or pronounced, resulting in inconsistencies in the association between age and telomere length across studies.

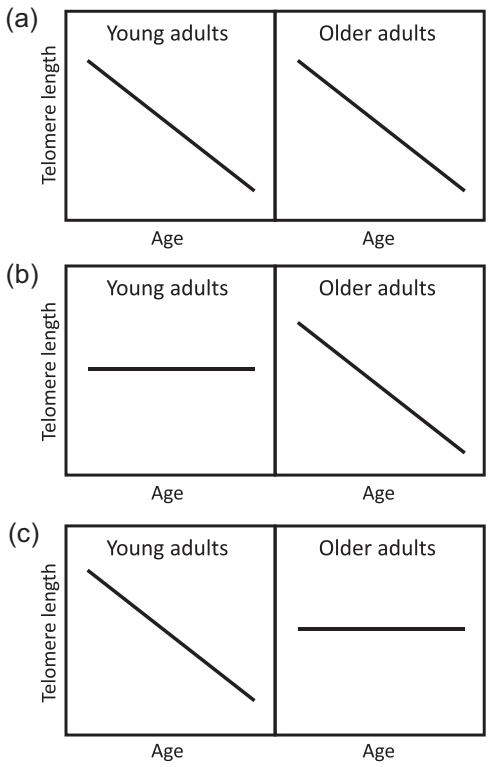

FIGURE 1 Expected associations between age and telomere length among young adults and older adults if (a) the rate of telomere shortening is constant across adult life, and (a) and (c) the rate of telomere shortening differs across the life course. (b) Telomeres are strongly protected during early adulthood but not later in life. (c) Telomeres are only protected once a length threshold has been reached. Note that this figure is a simplification and the association between age and telomere length can be more complex (e.g., nonlinear).

Here, we present an analysis of the association between telomere length (measured by TRF analysis of blood) and age in two groups of Japanese quail (*Coturnix japonica*) from the same captive breeding population that differ in their age distribution. The first group consisted exclusively of young adults, whereas the second group consisted of birds from a wide range of ages (including young adults, middle-aged and old individuals). If rates of telomere shortening are constant across an individual's adult life, we predict similar associations between age and telomere length in both age groups. Alternatively, if selection on self-maintenance differs across the life course, as proposed by evolutionary models of aging (Medawar, 1952; Williams, 1957) and the threshold model of telomere length maintenance (Aviv et al., 2015; Hasselquist & Tobler, 2021), we predict that the association between telomere length and age will be age-group specific (Figure 1).

2 | METHODS

2.1 | Breeding population

Individuals included in this study originated from a captive breeding population of Japanese quail (*C. japonica*) maintained at the University of Zurich. The breeding regime and husbandry procedures are described in detail in (Pick et al., 2016). For husbandry, males and females were brought into breeding cages inside our breeding facility for egg-laying. Eggs were artificially incubated, and chicks reared under standardized conditions. Once the birds were 4 weeks old, they were moved into large outdoor aviaries (females in single-sex aviary and males in mixed-sex aviary together with nonexperimental females) where their lifespan was recorded.

Within the framework of a long-term artificial selection experiment for divergent female reproductive investment (Pick et al., 2016), females were selected for breeding based on their egg investment. In short, the top 25% of females producing the largest versus smallest eggs relative to their body size were selected to breed for the first generation, and in the following generations the top 50% were used for breeding. After four generations of selective breeding, females of divergent breeding regimes differed in egg size by more than 1 SD, but they did not differ in the number of eggs laid (Pick et al., 2016;

Romero-Haro et al., 2023). Although the selection was female-limited, correlated responses in male reproductive success were observed (Pick et al., 2017). We will refer to males and females from the high and low reproductive investment regimes as high and low-line individuals, respectively.

Two cohorts of individuals, matched for breeding regime and sex, were randomly selected for the quantification of telomere length. In the first group sampled in 2015, 87 individuals of the fifth generation of the breeding experiment were included (44 females, 22 high and 22 low, and 43 males, 22 high and 21 low). All were young adults of a similar age (mean ± SE, range: females: 338 ± 7 days, 274–408 days; males: 341 ± 7 days, 274–408 days; $F_{1, 85} = 0.078$, $p = 0.781$). Hereafter, we will refer to this group as "young adults."

In the second group sampled in 2017, 82 individuals (41 females, 20 high and 21 low, and 41 males, 20 high and 21 low) of the 5th–8th generations of the breeding experiment and a wide range of ages were included (mean ± SE, range: females: 494 ± 38 days, 216–1018 days; males: 746 ± 38 days, 566–1018 days). Males were older than females in this group ($F_{1, 80} = 21.93$, $p < 0.001$). Individuals from this second group were killed for other research purposes, so it was not possible to record their natural lifespan. Hereafter, we will refer to this second group as "all-ages adults." Twenty individuals (five females and 15 males) from the first group were also included in the second group. These 20 individuals were 355 ± 10 days old (range: 275–408 days) when included in the "young adult" group and 965 ± 10 days old (range: 885–1018 days) when included in the "all-ages adults" group.

Individuals were blood sampled (~100 μL) from the brachial vein using heparinized capillary tubes when taken into the breeding facility for breeding. Blood samples were stored in the buffer (90% *N*-bromosuccinimide, 10% dimethylsulfoxide) at −80°C until analysis.

2.2 | Telomere length analyses

Because interstitial telomeres are present in quail (Stier et al., 2020), TRF analysis without DNA denaturation was used to quantify terminal telomere sequence length as described in Romero-Haro et al. (2022) (young adults) and Salomons et al. (2009) (all-ages adults). In short, extracted DNA was digested with restriction enzymes. The restricted DNA was then separated by pulsed-field gel electrophoresis and hybridized overnight with a ^{32}P-labeled telomere-specific probe (5′-CCCTAA-3′)x4. The radioactive signal of the telomere length distribution was detected by a phosphor screen and visualized using a phosphor imager. Densitometry in ImageJ (Salomons et al., 2009) was used to determine the position and strength of the radioactive signal compared with ^{32}P-labeled molecular markers to calculate telomere lengths for each sample. Telomere length was obtained within 0.5–48.5 kb and the average length for each individual was calculated. While telomere signal exists above 48.5 kb in this species, this signal is likely from class III telomeres (Delany et al., 2000), which are ultralong telomeres that do not change much with age (Delany et al., 2000). Samples were run in 10 gels for young adults and in 12 for all-ages adults. One golden sample was run on each gel showing a small intergel coefficient of variation (4.1% for young adults and 5.2% for all-ages adults). TRF analyses failed in four samples of young adults and one sample of an all-ages adult. The young adult and all-ages adult groups were analyzed in different years and using slightly different protocols (see Supporting Information materials for a full description of protocols) and telomere length values were therefore standardized within groups before analysis. An example of a TRF gel from each group is shown in Figure S1.

2.3 | Statistical analyses

We ran linear mixed effect models using the *R* package *lme4* (Bates et al., 2015) in *R* version 4. 1.2. (R Core Team, 2017) to test for an association between age and telomere length in the two groups (i.e., young adults vs. all-ages group). The interaction between group and age was included to specifically test if the association between age and telomere length differed between groups. Sex, breeding regime and the interaction between group and breeding regime were included as additional factors, and gel identity was included as a random effect to account for among-gel variation.

We ran additional analyses to test if the differences in the association between age and telomere length in the two groups (see Section 3) can be explained by telomere length-dependent selective disappearance or lifespan variation. To this end, we ran linear models to test for an association between telomere length as a young adult and lifespan. Breeding regime and sex were included as additional factors. Second, we ran generalized linear models with a binomial error structure to test if telomere length as a young adult affected the probability to be included in the all-ages group. Breeding regime and sex were included as additional factors. Finally, we used a linear model to test if telomere length as a young adult predicts telomere length as an old adult for the individuals included in both groups.

The significance of predictors in mixed models was determined by comparing two nested models with and without the factor of interest using likelihood ratio tests. For linear models, normality of the residuals and homoscedasticity were confirmed.

The data that support the findings of this study are available as Supporting Information material.

3 | RESULTS

We observed a significant interaction effect between group and age ($\chi^2 = 13.42$, $p < 0.001$; Table S1) on telomere length, showing that the association between age and telomere length differed between the two groups.

Telomeres significantly shortened with age in young adults ($\chi^2 = 16.55$, $p < 0.001$; Figure 2a, Table S2), but no significant association between telomere length and age was observed in the

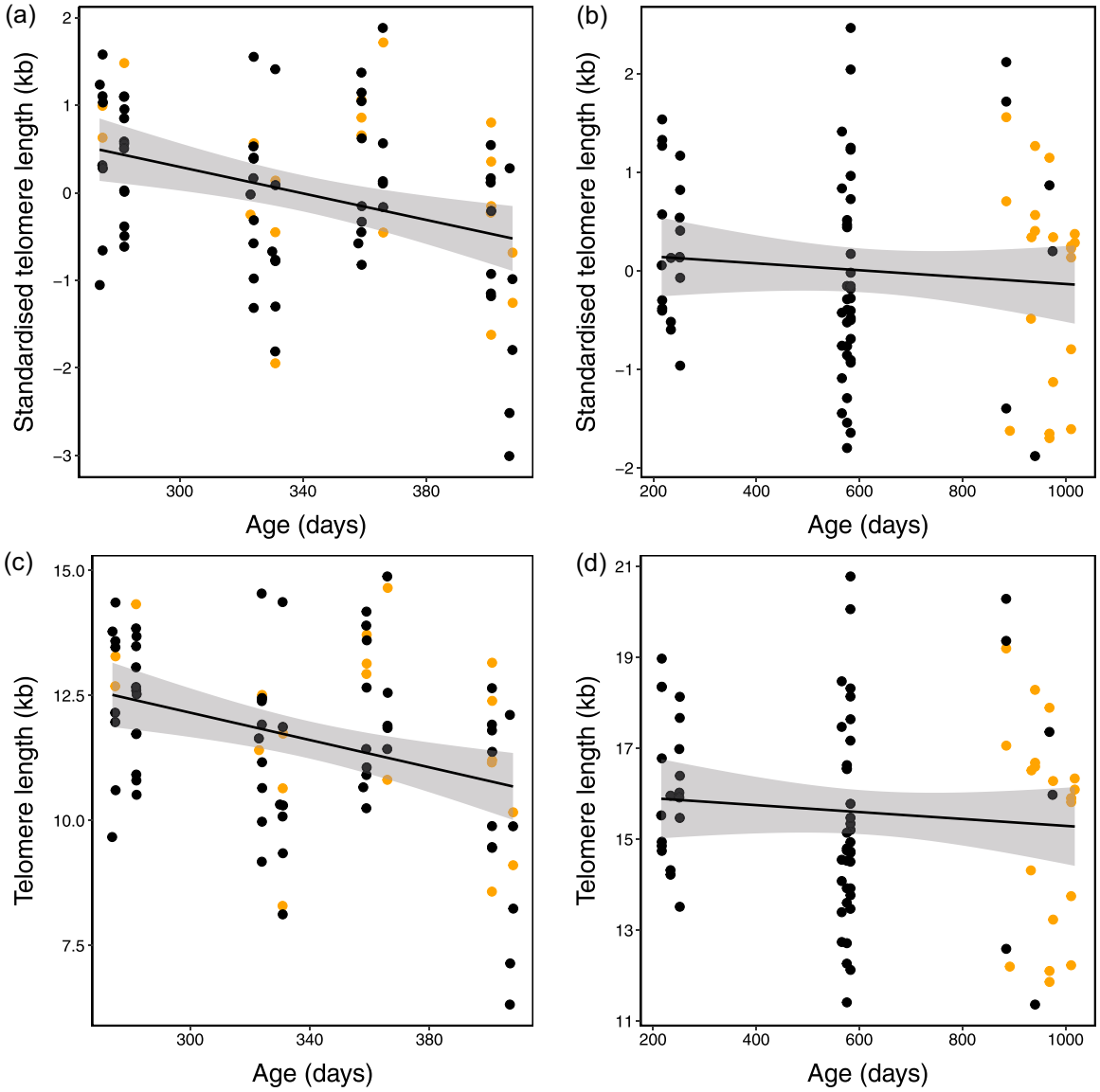

FIGURE 2 Association between age and standardized and raw telomere length among (a) and (c) young adults ($N = 83$) and (b) and (d) all-ages adults ($N = 81$), respectively. Regression lines and 95% confidence intervals are shown. Note that the association is significant in Panels (a) and (c), but not in Panels (b) and (d). Datapoints of individuals for which telomere length was quantified in both groups are shown in orange.

all-ages group ($\chi^2 = 1.630$, $p = 0.202$; Figure 2b, Table S2). Similarly, the effect of the breeding regime on telomere length was opposite in the two groups ($\chi^2 = 26.94$, $p < 0.001$; Table S1, Figure S2). Low-line individuals showed longer telomeres than high-line individuals in the young adults group ($\chi^2 = 21.05$, $p < 0.001$; Table S2), but shorter telomeres in the all-ages group ($\chi^2 = 8.523$, $p = 0.004$; Table S2). Analyzing raw data instead of standardized telomere lengths did not change results qualitatively (see Figure 2c,d, Tables S3 and S4).

Telomere length as a young adult did not predict lifespan ($F_{1,\ 77} = 2.982$, $p = 0.088$; Table S5) and telomere length as a young adult did not influence the probability of being included in the all-ages group (i.e., probability of being measured a second time, $\chi^2 = 0.042$, $p = 0.838$; Table S6). Thus, telomere length-dependent selective disappearance cannot explain the differences in the association between age and telomere length observed between groups. Furthermore, telomere length as a young adult did not predict telomere length as an old adult in the subset of 20 individuals included in both groups ($b = -0.369$, SE = 0.219, $F_{1, 18} = 2.832$, $p = 0.109$; Figure S3).

4 | DISCUSSION

Here, we show that the association between age and telomere length is age-dependent. Whereas telomere length was strongly negatively associated with age during early adulthood, this association disappeared when a wider range of ages were included. This age-specific association may partly explain the discrepancies in patterns

of age-dependent telomere length and shortening observed among studies (Remot et al., 2021).

Telomere length shortens with each cell division (Harley et al., 1992), and the negative association between telomere length and age in early adulthood thus makes mechanistic sense. The age-related shortening is no longer found among older individuals. These results are inconsistent with evolutionary models of aging, which predict selection for self-maintenance to be stronger early in life (Kirkwood, 1977; Medawar, 1952; Williams, 1957). The observed pattern is, however, consistent with a threshold model of telomere maintenance (Aviv et al., 2015; Hasselquist & Tobler, 2021; Tobler et al., 2022) (Figure 1c), suggesting that older individuals prevent further telomere attrition through telomere stabilization or elongation once telomeres are critically short. Importantly, mortality is very low until young adulthood in our population and telomere length as a young adult did neither predict an individual's lifespan, nor it is probability to be included in the "all-ages adults group" (i.e., to be measured a second time). Thus, selective disappearance cannot explain the observed patterns. Furthermore, it is unlikely that the slight differences in TRF protocols generated biases in our data. Telomere length was analyzed within the same range (0.5–48.5 kb) for both cohorts and results were qualitatively unchanged when analyzing standardized telomere length (i.e., controlling for among-cohort variation) or using raw telomere length data (Figure 2).

Our findings suggest that costs and benefits of having short telomeres may change throughout an individual's life. Selection for telomere length stabilization appears to be relaxed in young adults, either because the costs of telomere shortening are small compared to the costs of telomere stabilization, and/or because shorter telomeres might have direct benefits in this age group (e.g., reduced risk of cancer [Young, 2018]). Once telomeres reach a critical length, however, individuals may need to prevent further shortening, and typically this minimal length may only be reached at middle to old age. The mechanisms by which older individuals prevent further telomere shortening are currently unknown but may include increased antioxidant defense (Reichert & Stier, 2017), telomerase activity (Greider & Blackburn, 1985) or other telomere stabilization or elongation mechanisms (Lundblad, 2002).

It has been assumed that including a wide range of ages in cross-sectional studies will increase the probability to find a negative association between telomere length and age (Remot et al., 2021; Tricola et al., 2018). However, our results show that this might not necessarily be the case because of different processes and selection pressures acting at different life stages (see also Angelier et al. [2019], Hall et al. [2004], Hoelzl et al. [2016]), and indeed age-specific associations between telomere length and age might explain the lack of an association in previous studies that focused on middle-aged and old individuals (e.g., Angelier et al., 2019; Hall et al., 2004; Hoelzl et al., 2016). Similarly, an overrepresentation of young individuals (e.g., Asghar et al., 2015; Cherdsukjai et al., 2020) might lead to an overestimation of the strength of association between telomere length and age across the life course.

These age-specific patterns of telomere attrition and protection were further confirmed when comparing birds from the different breeding regimes: early in life, individuals that invested heavily in reproduction paid a cost in terms of accelerated telomere attrition (see also Romero-Haro et al. [2022]). In older individuals, however, this pattern reversed, suggesting that life history strategies influence age-specific patterns of telomere maintenance (Gómez-Blanco et al., 2023; Marasco et al., 2022).

In conclusion, we show that the association between telomere length and age is age-dependent, potentially explaining the large heterogeneity in patterns of telomere length—age associations observed across studies. These findings suggest that the costs and benefits associated with telomere maintenance are dynamic across an individual's life course.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information material of this article.

ETHICS STATEMENT

All procedures complied with all relevant ethical regulations and were conducted under licenses provided by the Veterinary Office of the Canton of Zurich, Switzerland (permits 195/2010, 14/2014, 156) and the ethical committee of the University of Exeter (permit eCORN002475).

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SUPPORTING INFORMATION

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

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