Article:
van Lieshout, SHJ orcid.org/0000-0003-4136-265X, Bretman, A orcid.org/0000-0002-4421-3337, Newman, C et al. (3 more authors) (2019) Individual variation in early-life telomere length and survival in a wild mammal. Molecular Ecology, 28 (18). pp. 4152-4165. ISSN 0962-1083

https://doi.org/10.1111/mec.15212

Individual variation in early-life telomere length and survival in a wild mammal

Sil H.J. van Lieshout[1,2], Amanda Bretman[1], Chris Newman[3], Christina D. Buesching[3], David W. Macdonald[3] & Hannah L. Dugdale[1]

[1]School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK; [2]NERC Biomolecular Analysis Facility, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK; [3]Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, The Recanati-Kaplan Centre, Abingdon, Oxfordshire OX13 5QL, UK

Correspondence author: Sil H.J. van Lieshout

E-mail: sil.vanlieshout@gmail.com

ORCID: SHJvL, 0000-0003-4136-265X; AB, 0000-0002-4421-3337; CN, 0000-0002-9284-6526; CDB, 0000-0002-4207-5196; DWM, 0000-0003-0607-9373; HLD, 0000-0001-8769-0099

Abstract

Individual variation in survival probability due to differential responses to early-life environmental conditions is important in the evolution of life-histories and senescence. A biomarker allowing quantification of such individual variation, and which links early-life environmental conditions with survival by providing a measure of conditions experienced, is telomere length. Here, we examined telomere dynamics among 24 cohorts of European badgers (*Meles meles*). We found a complex cross-sectional relationship between telomere length and age, with no apparent loss over the first 29 months, but with both decreases and increases in telomere length at older ages. Overall, we found low within-individual consistency in telomere length across individual lifetimes. Importantly, we also observed increases in telomere length within individuals, which could not be explained by measurement error alone. We found no significant sex differences in telomere length, and provide evidence that early-life telomere length predicts lifespan. However, while early-life telomere length

predicted survival to adulthood (≥1 year old), early-life telomere length did not predict adult survival probability. Furthermore, adult telomere length did not predict survival to the subsequent year. These results show that the relationship between early-life telomere length and lifespan was driven by conditions in early-life, where early-life telomere length varied strongly among cohorts. Our data provide evidence for associations between early-life telomere length and individual life-history, and highlight the dynamics of telomere length across individual lifetimes due to individuals experiencing different early-life environments.

Keywords: telomere length, early-life conditions, biomarker, senescence, wild population, mammal

1. Introduction

Species from most taxa exhibit a loss of performance in later-life that increases the probability of mortality (Medawar 1952; Williams 1957). This process of senescence is common, but highly variable across taxa (Jones *et al.* 2014) and even within species (Campbell *et al.* 2017; Dugdale *et al.* 2011; Nussey *et al.* 2009). Pioneering laboratory studies using controlled environments have provided important insights into senescence patterns, but cannot explain the remarkable variation in the onset and rate of senescence in wild populations, where selection acts under naturally varying conditions (Partridge & Gems 2007). Hence, studies of wild populations have informed understanding of how early-life environments shape individual senescence patterns (Cooper & Kruuk 2018; Lemaitre *et al.* 2015; Nussey *et al.* 2013). This understanding has been further improved by quantification of extrinsic effects through biomarkers that reflect ecological effects that are otherwise difficult to measure (Bebbington *et al.* 2016; Spurgin *et al.* 2017).

Telomere length, which reflects the physiological consequences of within-individual experiences and facilitates between-individual comparisons, is a biomarker of senescence (Monaghan & Haussmann 2006). Telomeres are non-coding hexameric repeats (5'-TTAGGG-3') that, with

51 associated shelterin proteins, prevent end-to-end fusion of linear chromosomes and maintain genomic
52 integrity (Blackburn 2000; de Lange 2004). Telomeres shorten with age due to incomplete DNA-
53 replication at the 3'-end of the DNA-strand (Olovnikov 1973). This occurs more rapidly in early-life due
54 to higher levels of cellular division during growth (Frenck *et al.* 1998; Hall *et al.* 2004), or in response
55 to metabolically demanding activities (e.g. reproduction; Heidinger *et al.* 2012; coping with
56 stress/disease; Epel *et al.* 2004; Willeit *et al.* 2010). The amount of telomeric DNA lost in each cell
57 division depends on cellular conditions (Monaghan & Ozanne 2018) and oxidative stress (Reichert &
58 Stier 2017; von Zglinicki 2002; but see Boonekamp 2017). Telomeres can, however, be replenished by
59 telomerase, the telomere-elongating enzyme (Blackburn *et al.* 1989). Telomerase is transcriptionally
60 repressed later in development (Blackburn *et al.* 1989), but alternative pathways for telomere
61 lengthening do exist (Cesare & Reddel 2010; Mendez-Bermudez *et al.* 2012). Telomere shortening
62 occurs until cells enter a state of arrest, inducing replicative senescence, where the accumulation of
63 senescent cells, due to progressive loss of regenerative capacity (Campisi & di Fagagna 2007), can
64 impair tissue functioning (Armanios & Blackburn 2012; Campisi 2005).

65 Variation in the rate of telomere shortening occurs among organisms (Monaghan 2010). For
66 example, mean human leukocyte telomere length shows a biphasic decline with age, with rapid
67 shortening in early-life followed by slower attrition in adulthood (Aubert & Lansdorp 2008).
68 Correlations among within-individual telomere measurements in humans were high (0.82 – 0.93;
69 Benetos *et al.* 2013), which corroborates the high individual repeatability (i.e. 81 – 83%) in telomere
70 length in wild populations using TRF (telomere restriction fragment) methods (Bauch *et al.* 2013;
71 Boonekamp *et al.* 2014). However, longitudinal studies in wild populations using a qPCR (quantitative-
72 PCR) approach across individual lifetimes reported much lower (i.e. 7 – 13%) individual repeatability in
73 telomere length (Fairlie *et al.* 2016; Spurgin *et al.* 2017), indicating that telomeres are highly dynamic
74 over individual lifetimes. Indeed, telomere length can both decrease and increase with age (Bateson
75 & Nettle 2016), which has been attributed to measurement error (Steenstrup *et al.* 2013) but cannot

be explained by measurement error alone (Spurgin *et al.* 2017). Telomere length can therefore exhibit complex relationships with age, explained by within-individual changes, and provide a measure of conditions experienced that links to individual life-history.

Telomere length has been linked positively to survival to adulthood and/or annual adult survival probability in both captive (Heidinger *et al.* 2012) and wild populations (Asghar *et al.* 2015b; Barrett *et al.* 2013; Cram *et al.* 2017; Fairlie *et al.* 2016; Haussmann *et al.* 2005). Even though other studies have tested for, but not found such associations (Beaulieu *et al.* 2011; Sudyka *et al.* 2014), a meta-analysis in non-human vertebrates reported an overall association between short telomeres and higher mortality risk (Wilbourn *et al.* 2018). While this provides evidence for a link between telomere length and life-history, whether telomere length plays a direct causal role in senescence, because telomeres are integral to organismal function, or acts as a non-causal biomarker of somatic integrity remains currently unclear (Simons 2015; Young 2018).

Compelling evidence exists that early-life conditions such as maternal effects, developmental stress and competition for resources (e.g. Asghar *et al.* 2015a; Haussmann *et al.* 2012; Cram *et al.* 2017) can be particularly influential in shaping telomere length. The greater strength of early-life than late-life effects could be due to stronger forces of selection, since natural selection acts on the proportion of a cohort that is alive, which is greatest in early-life (Hamilton 1966). However, greater selection in early-life is affected by a trade-off between parental and offspring survival (Lee 2008; Lee 2003), causing the evolutionary paradigm around early-life telomere length to remain relatively poorly understood (Vedder *et al.* 2017). Nevertheless, early-life telomere length might be an important predictor of life-histories (Monaghan 2010; Wilbourn *et al.* 2018; Young 2018). While studies into the effects of the environment on telomeres are emerging in wild mammals (Cram *et al.* 2017; Izzo *et al.* 2011; Lewin *et al.* 2015), longitudinal studies in wild mammals remain relatively rare (Beirne *et al.* 2014; Fairlie *et al.* 2016). Gaining a better understanding of telomere dynamics, its relationship with survival, and early-life effects requires more comprehensive longitudinal studies in wild populations.

The European badger (*Meles meles*; henceforth 'badger') provides an informative mammalian model species for studying the effects of early-life conditions on telomere length and senescence patterns. We benefit here from a long-term study of badgers at Wytham Woods (Oxford, UK; Macdonald *et al.* 2015); an almost closed population (see Macdonald *et al.* 2008) with a high and relatively consistent annual recapture rate of 84% (SE = 1.3%; Macdonald *et al.* 2009) over 1726 life-histories monitored seasonally since 1987. In this population, badgers live in polygynandrous social groups (mean group size: 11.3, range: 2 – 29; da Silva *et al.* 1994; Macdonald *et al.* 2015), and show reproductive senescence (Dugdale *et al.* 2011). Badgers have one litter per year (mean litter size 1.4 ± 0.06 SE; range 1 – 4; Dugdale *et al.* 2007), where cubs emerge from underground dens at 6 – 8 weeks of age, are weaned at 12 weeks, and reach independence at 14 – 16 weeks old (Fell *et al.* 2006). Cub survival probability ranges from 61 – 94% (mean ± SE = 67% ± 3%; Macdonald *et al.* 2009), and cub cohorts are negatively impacted by early-life exposure to endo-parasitic coccidia infection (Newman *et al.* 2001), oxidative stress (Bilham *et al.* 2018) and unseasonable weather variation (Macdonald *et al.* 2010; Noonan *et al.* 2014; Nouvellet *et al.* 2013). We therefore posit that strong selection pressures on badger cubs may be reflected in their telomere length and survival probability.

Here, we investigate longitudinal telomere dynamics among 24 cohorts in wild badgers. Relative leukocyte telomere length (RLTL) measurements were used to test: (i) age-related variation in RLTL and the extent to which this was driven by within-individual changes, and both cohort and sex effects; (ii) the repeatability of RLTL and whether within-individual changes in telomere length are attributed to measurement error; and (iii) whether early-life and adult RLTL predict survival and lifespan.

2. Methods

2.1 Study system

We conducted this study in Wytham Woods, Oxfordshire, UK (51°46'24"N, 1°20'04"W), a 424 ha mixed semi-natural woodland site surrounded by mixed arable and permanent pasture (Macdonald & Newman 2002; Macdonald *et al.* 2004; Savill 2010). The resident high-density badger population (range = 20.5 – 49.5 badgers/km^2; Macdonald *et al.* 2015) forms large social groups (Johnson *et al.* 2000). Badger social groups have clearly demarcated territories (Buesching *et al.* 2016; Delahay *et al.* 2000), although badgers do cross these borders when foraging and meet amicably with neighbouring groups (Ellwood *et al.* 2017; Noonan *et al.* 2015). Mean annual adult survival rates in this population are 0.83 (± 0.01 SE, Macdonald *et al.* 2009) with a mean adult lifespan of 4.96 years (± 3.21 SD; Bright Ross, J., Pers. Comm.).

Trapping has been undertaken three or four times per year since 1987, for two to three consecutive days per social group. Trapped badgers were anaesthetised using an intra-muscular injection of 0.2 ml ketamine hydrochloride per kg body weight (McLaren *et al.* 2005) and identified by a unique tattoo number on the left inguinal region. Capture date, sett, social group (comprising several setts, i.e. burrow systems), sex, age-class (cub <1 year; adult ≥1 year) and morphometric measurements (i.e. length, weight, tooth wear; da Silva & Macdonald 1989; Macdonald *et al.* 2009) were recorded for each badger. Badger age was defined as the number of days elapsed since the 14th of February in their respective birth year (reflecting the February birth peak; Yamaguchi *et al.* 2006) . Blood was collected by jugular venipuncture into vacutainers with an EDTA anticoagulant, and stored at -20°C immediately. Badgers were released at their setts, after full recovery from anaesthesia.

2.2 Telomere analyses

We selected 1248 blood samples from 612 individuals, representing 308 males and 304 females, comprising individuals varying in lifespan (range: 14 – 233 months; mean ± SE = 97.2 ± 1.88 months) and from different cohorts (*n* = 24). Only badgers for which age could be determined, either trapped as a cub (*n* = 545) or inferred through low tooth wear, were included (*n* = 67; males = 26, females = 41;

tooth wear 1 indicates a cub and tooth wear 2 indicates a 1-year old adult (da Silva & Macdonald 1989; Macdonald *et al.* 2009), where young individuals also had to have length <685 mm and weight <8 kg). Individuals were either sampled once (*n* = 163) or more (*n* = 449 badgers; 2 – 9 times per individual) for telomere length analyses. Only badgers which were considered dead at the time of analysis were included. All analyses were also run without the 67 individuals for which age was determined through tooth wear, to confirm that inclusion of these samples did not bias the results (see supporting results S1).

Genomic DNA was extracted from whole blood using the DNeasy Blood & Tissue kit (Qiagen, Manchester, UK) according to the manufacturer's protocol, with adjustments using 125 µl of anticoagulated blood and a double elution step (2x 75 µl AE buffer). DNA integrity was assessed by running a random selection of DNA extracts (ca. 20%) on agarose gels to check for high molecular weight. DNA concentration of all samples was quantified using the Fluostar Optima fluorometer (BMG Labtech, Ortenberg, Germany) and standardized to 20 ng/µl, after which samples were stored at -20 °C.

Relative leukocyte telomere length (RLTL) measurements were made using the monochrome multiplex qPCR method described by Cawthon (2009). This method provides a ratio of the abundance of telomeric sequence to that of the control gene IRBP, the T/S ratio, analysed in the same well which should reduce measurement error by excluding pipetting errors and well effects. DNA samples were assayed using SYBR® Select Master Mix (Applied Biosystems, Warrington, UK) with telomere primers telg (5'-ACA-CTA-AGG-TTT-GGG-TTT-GGG-TTT-GGG-TTT-GGG-TTA-GTG-T-3') and telc (5'-TGT-TAG-GTA-TCC-CTA-TCC-CTA-TCC-CTA-TCC-CTA-TCC-CTA-ACA-3') at a concentration of 900 nM. A GC-clamp was added to the control gene (inter-photoreceptor retinoid-binding protein; IRBP) primers to allow for sufficiently different melt temperatures between the control gene and telomeric sequences, using GC-clamped IRBP primers IRBP-F (5'-CGG-CGG-CGG-GCG-GCG-CGG-GCT-GGG-CGG-GCC-ACA-TTT-CTG-GTA-TCC-CCT-3') and IRBP-R (5'-GCC-CGG-CCC-GCC-GCG-CCC-GTC-CCG-CCG-GGG-CGG-TCG-TAG-ATG-

GTA-TC-3') at a concentration of 900 nM. Subsequent melt-curve analysis confirmed differential melt-curves and lack of primer-dimer formation. Semi-skirted 96-well polypropylene qPCR plates were loaded manually with initial reaction volumes of 20 µl. Each well contained 10 µl of SYBR® Select Master Mix (Applied Biosystems, Warrington, UK), 4.9 µl of nuclease free water, 0.9 µM of both the forward and reverse primers (900 nM) and 1.5 µl of 20 ng/µl DNA sample (which was replaced with 1.5 µl of nuclease free water in controls) and sealed with PCR-plate film adhesive. Cycling conditions in the Quantstudio 12K flex real-time PCR system (Applied Biosystems, Warrington, UK) were: 50°C for 2 min and 95°C for 2 min, followed by 2 cycles at 94°C for 15 sec and 49°C for 15 sec, then 40 cycles at 94°C for 15 sec, at 60°C for 10 sec, at 74°C for 15 sec, at 84°C for 10 sec and 86°C for 15 sec. A serially diluted (4x from 80 to 0.3125 ng/µl) 'reference' sample was included on each qPCR plate to produce a standard curve to calculate plate efficiencies, where the 20 ng/µl dilution was used as a calibrator. The reference sample was collected from a badger in 2005 and was subject to the same capture methods and long-term storage as the other samples that we analysed.

Samples were randomly allocated to qPCR plates and run in duplicate in adjacent wells, after which amplicon lengths and telomeric sequences were confirmed on the Agilent TapeStation 4200 and 3730 DNA Analyzer (Applied Biosystems, Warrington, UK) with the Big Dye 3.1 cycle sequencing kit (Applied Biosystems, Warrington, UK). Cq-values on the 34 qPCR plates declined in a log-linear fashion (r^2>0.99). Using LinRegPCR 2017.1 (Ruijter *et al.* 2009) we corrected for baseline fluorescence, determined the windows of linearity for the amplification curves (0.432 for IRBP and 0.694 for telomeres) and calculated efficiencies and Cq-values for each well. Reaction efficiencies were (mean ± SE) 1.793 ± 0.004 for IRBP and 1.909 ± 0.004 for telomeres, and we calculated RLTL according to Pfaffl (2001):

$$RLTL = \frac{(E_{tel}\text{^}(Cq_{tel(calibrator)} - Cq_{tel(sample)}))}{(E_{IRBP}\text{^}(Cq_{IRBP(calibrator)} - Cq_{IRBP(sample)}))}$$

where E_{tel} and E_{IRBP} represent the mean well efficiencies for each of the amplicons, $Cq_{tel(calibrator)}$ and $Cq_{IRBP(calibrator)}$ are the mean Cq-values for the calibrators (20 ng/µl) for each amplicon and $Cq_{tel(sample)}$ and $Cq_{IRBP(sample)}$ are the mean Cq-values for both amplicons in each sample.

Inter-plate repeatability (intraclass correlation coefficient), calculated with rptR 0.9.2 (Stoffel *et al.* 2017), was calculated with the reference sample by comparing variance among duplicates of the reference sample within a plate, to variance of the reference sample among plates and estimated at 0.82 (95% CI = 0.76 – 0.87; *n* = 142 samples; 34 plates). Intra-plate repeatability was calculated with duplicates of the same sample on the same plate, while controlling for plate effects, and estimated at 0.90 (95% CI = 0.86 – 0.93; *n* = 1248 samples; 34 plates) for IRBP, 0.84 (95% CI = 0.79 – 0.90; *n* = 1248 samples; 34 plates) for telomere Cq-values and 0.87 (95% CI = 0.82 – 0.91; *n* = 1248 samples; 34 plates) for RLTL measurements (for further details on quality control see supporting methods).

2.3 Statistical analyses

Statistical analyses were conducted in R 3.3.1 (R Development Core Team 2019), with RLTL measurements square-root transformed to meet the assumptions of Gaussian error distributions in models with RLTL as the response variable.

2.3.1 Age, sex and cohort effects on telomere length

We assessed the relationship between RLTL and age (months), and the interaction with cohort, following Fairlie *et al.* (2016) and Spurgin *et al.* (2017). We tested a variety of age functions in General Linear Mixed Models (GLMMs; Bates *et al.* 2015) that included individual ID, plate ID and year as random effects, and sex, sample storage time (months), and in some models cohort, as fixed effects. We checked for collinearity and found that sample storage time and cohort were collinear (VIF>3), since sample storage time is similar within cohorts. We therefore first determined that sample storage time was not associated with telomere length (β = -0.006 ± 0.010 SE, X^2 = 0.383, d.f. = 1, P = 0.536) and

then excluded it from subsequent models. We considered a null model (without the age terms), polynomial age terms (linear, quadratic, cubic), a full-factorial age term and a variety of threshold functions. Visual inspection of the data indicated inflection points, with further specification of inflection points through comparison of AIC values, at 29, 65 and 112 months of age. These threshold models (with either a single, double or triple threshold) were compared to all other models. We ran additional models to test whether adding a cohort fixed effect and an interaction between age and cohort improved the model, using AIC values. We did not fully apply model selection or averaging, as we aimed to compare a set of specifically defined models, where the model with the lowest AIC fits these data best, but we considered all plausible models with ΔAIC <7.

We then tested age-specific sex differences in telomere length through an interaction between age and sex in the best fitting age model and all non-significant interactions were dropped. In the same model we included age at last capture (α_i), as a measure of lifespan (van de Pol & Verhulst 2006), to test if selective disappearance of individuals contributed to the age pattern observed. We also compared, in the same model, within-individual (β_W) to between-individual (β_B) slopes, where the difference between these slopes is exactly the effect of selective disappearance (van de Pol & Verhulst 2006). In a separate model we tested the significance of the between-individual component by replacing age parameters by within-group deviation scores (age - α_i).

2.3.2 Individual repeatability and telomere elongation

Individual repeatability (across multiple samples from the same individual) was calculated by dividing the variance explained by individual identity by total phenotypic variance, in a Gaussian-distributed model (identity link function), across all samples (*n* = 1248) and only for adult samples (*n* = 779). These models included RLTL as the response variable and the best fitting age variable and cohort as fixed effects, with individual ID and qPCR-plate as random effects. The variance explained by qPCR-plate was then excluded from the total phenotypic variance as it is a source of experimental measurement error

and therefore not biologically relevant phenotypic variance; thus, it could lead to underestimation of repeatability (Dochtermann *et al.* 2015). Additionally, we determined the correlation between within-individual telomere measurements, using the marginal R^2 (Nakagawa & Schielzeth 2013), in a Gaussian-distributed model (identity link function) with RLTL as the response variable, RLTL at *t+1,* cohort and age (months) as fixed effects and individual ID as a random effect.

We examined increases in RLTL with age by estimating differences in telomere lengths among technical replicates, i.e. duplicates next to each other within a qPCR-plate, and among within-individual samples, i.e. difference in RLTL between within-individual samples. We used MCMCglmm (Hadfield 2010) with an inverse Wishart prior (v = 1, nu = 0.002), 600,000 iterations, a thinning of 300 and burn-in period of 15,000 iterations, to test whether within-individual changes in RLTL were greater than measurement error. We randomly selected two samples per individual, and built a model with telomere length as the response variable and individual ID and qPCR-plate as random effects (*n* = 898 samples; 449 individuals). We then randomly selected one set of duplicates per individual, and constructed a model with telomere length for each of the technical replicates as the response variable and individual ID as a random effect (*n* = 898 samples; 449 individuals). We compared the explained variance by the random effect for individual ID between these two models and whether the 95% credible intervals overlapped. Additionally, we separated the dataset into groups that either increased or decreased in RLTL and ran these models again for these groups separately. We also tested if the residual error variance ($\bar{\sigma}_{\varepsilon}^{2}$) was smaller than the error variance in RLTL, when RLTL can increase or decrease ($\sigma_{\varepsilon}^{'2}$), following Simons *et al.* (2014), which would reject the hypothesis that RLTL shows no elongation.

2.3.3 Telomere length, survival and lifespan

We used GLMMs to test the relationship between early-life RLTL (<1 year old) and lifespan (*n* = 435). In the following models, we conducted model averaging, using an information theoretic approach to

select plausible models and estimate the relative importance of fixed effects for models with ΔAIC <7 with the "natural average method" (Burnham *et al.* 2011). All four models included sex as a fixed factor, and plate and natal social group as random effects. Early-life RLTL did not vary with age (n = 435, β = -0.002 ± 0.006 SE, X^2 = 0.160, d.f. = 1, P = 0.690); therefore, age was not included in GLMMs with early-life RLTL as a fixed effect. Firstly, early-life RLTL as a predictor of lifespan was modelled with lifespan as the response variable (n = 435), including early-life RLTL and cohort as additional fixed effects in a Poisson-distributed model (log link function). We also controlled for overdispersion by including observation (for each unique measure) as a random effect (Harrison 2014). Lifespan was determined as the age at last capture. To ensure the different survival probabilities for cubs and adults did not alter the results we also ran a model (see Table S1) with lifespan calculated in months as the difference between the date of birth and last capture, with 24 months added when last captured as adults, due to a 95% recapture interval of 2 years (Dugdale *et al.* 2007), and 12 months as cub due to their different survival rates (Macdonald *et al.* 2009). Secondly, we modelled survival to adulthood (≥1 year old) using a binary term in a binomial (logit link function) mixed-effects model with early-life RLTL (n = 435) and cohort as additional fixed effects. Thirdly, we used a Cox mixed-effects model to test whether early-life RLTL predicts annual adult survival probability over the lifetime of individuals that survived their first year. The model included early-life RLTL (n = 336) as an additional fixed effect, and cohort as an additional random effect. Finally, we tested the relationship between adult RLTL (n = 779) and survival to the subsequent year, in a binomially-distributed model (logit link function) with RLTL interacting with age (based on the best fitting model) as an additional fixed effect and individual ID (correcting for multiple measures per individual), cohort, current social group and year as additional random effects.

3. Results

3.1 Age, sex and cohort effects on telomere length

Across all samples, after no change up to and including 29 months of age, RLTL increased up to and including 65 months, followed by a decline up to and including 112 months, with a second increase in RLTL in older age (Table 1; Figure 1). Two models had ΔAIC <7, with the top model including all thresholds, and the second-best model with thresholds at 65 and 112 months, where both models included a fixed factor for cohort (Table S2 and Figure S1). Males and females had similar telomere lengths (Table 1) and there was no evidence for different age patterns by sex. Cohorts from earlier years (1987 – 1992) had lower and more variable early-life RLTL measurements than those from subsequent years (Figure 2a). We thus repeated these analyses where these cohorts were omitted, which showed that these cohorts did not alter the results (see supporting results S2).

Selective disappearance of individuals was accounted for by including age at last capture (β_S) in the best fitting age model, which was borderline significant (Table 1). However, there was a between-individual effect (β_B) and a within-individual effect (β_W) for individuals aged 29 months or older, where the difference between these slopes is due to selective disappearance of individuals with shorter telomeres (Table 1). Consequently, selective disappearance of individuals with shorter telomeres did contribute to the age pattern observed.

3.2 Individual repeatability and telomere elongation

Individual repeatability was 0.017 (95% CI = 0.001 – 0.098) including cub and adult RLTL estimates, and 0.026 (95% CI = 0.001 – 0.143) using only RLTL measurements from adulthood. These repeatabilities changed to 0.022 (95% CI = 0.001 – 0.103) and 0.039 (95% CI = 0.001 – 0.154), respectively, when plate variance (measurement error) was removed from the phenotypic variances, so 2.2% of the variance in RLTL was explained by within-individual consistency among samples. There was no significant correlation between RLTL measured at different time points in the same individual (marginal R^2 = 0.067; X^2 = 0.92, P = 0.336; Figure 2b).

Increases (in the range of 0.004 – 5.829% per month) in RLTL were identified in 61.2% of within-individual changes (Figure 2c) for individuals with ≥2 samples (n = 449). When accounting for plate effects using MCMCglmm, the random effect estimate for individual ID with technical replicates was 0.0331 (95% CI = 0.0290 – 0.0376), whereas for within-individual samples the random effect estimate was 0.0014 (95% CI = 0.0003 – 0.0044; Figure 2d). For the group that exhibited increases in RLTL the random effect estimate for individual ID with technical replicates was 0.0345 (95% CI = 0.0289 – 0.0424), whereas for within-individual samples this estimate was 0.0016 (95% CI = 0.0003 – 0.0058). The random effect estimate for technical replicates in the group that exhibited decreases in RLTL was 0.0359 (95% CI = 0.0310 – 0.0452) and for within-individual samples this estimate was 0.0006 (95% CI = 0.0003 – 0.0045), where none of the 95% credible intervals from the technical replicates and within-individual samples overlapped. Additionally, residual variance among samples was smaller ($\bar{\sigma}_{\varepsilon}^{2}$ = 0.041) than the overall change in RLTL ($\sigma_{\varepsilon}^{'2}$ = 0.922; $F_{31,40}$ = 22.48, P <0.001). These within-individual increases in RLTL were therefore not solely due to measurement error.

3.3 Telomere length, survival and lifespan

Early-life RLTL (<1 year old) was positively associated with lifespan (Figure 3 and 4a; Table S3 and S4), where individuals with longer telomeres in early-life had longer lifespans, such that an increase of 1 T/S ratio was associated with 13.3% greater longevity. However, this association was underpinned by survival benefits in early-life and not in adulthood as early-life RLTL only predicted survival to adulthood (Figure 5 and 4b; Table S4 and S5). In contrast, early-life RLTL showed no relationship with annual adult survival probability (Table S4) and adult RLTL showed no association with survival to the subsequent year (Figure 4c; Table S4 and S6), but all models indicated an effect of cohort on survival and lifespan (Figure S2; Table S4).

4. Discussion

We found complex telomere dynamics with no apparent change (≤29 months of age), decreases (i.e. between 65 and 112 months) and increases in RLTL with age (>29 and ≤65, and >112 months). This pattern was mainly due to within-individual changes. However, selective disappearance of individuals with shorter telomeres contributed to the age pattern observed when age at last capture was included (as a measure of selective disappearance) and within- and between-individual slopes were compared. While the lack of change in RLTL in early-life contrasts with previous studies that have reported rapid declines in RLTL with age in early-life (Aubert & Lansdorp 2008; Baerlocher *et al.* 2003), we are unable to sample individuals until at least 3 months of age, due to welfare legislation (Protection of Badgers Act, 1992), and therefore we may miss the period where the greatest changes in RLTL occur. The combination of selective mortality and within-individual changes in RLTL was also reported in wild Soay sheep (*Ovis aries*; Fairlie *et al.* 2016), providing evidence for complex relationships between telomere length and age.

Male and female badgers had similar telomere lengths across all ages, corroborating recent findings in wild meerkats (*Suricata suricatta*) and European badgers in Woodchester (Beirne *et al.* 2014; Cram *et al.* 2017), but contrasting with age-specific sex differences in telomere length in Soay sheep (*Ovis aries*; Watson *et al.* 2017). The lack of age-specific sex differences in badgers and meerkats could be due to males and females having similar lifespans, whereas in Soay sheep females live much longer than males (Cram *et al.* 2017; Fairlie *et al.* 2016; Macdonald & Newman 2002).

Individual repeatability in RLTL was only 2.2% throughout an individual's lifespan. The point estimate was higher (3.9%) when only including RLTL measurements in adulthood, but the 95% confidence intervals overlapped greatly, and within-individual RLTL measurements were not correlated. Within-individual RLTL correlations in humans were high (0.82 – 0.93; Benetos *et al.* 2013) and individual repeatability in RLTL in avian TRF studies was also high (81% – 83%; Bauch *et al.* 2013; Boonekamp *et al.* 2014). In contrast, lifelong qPCR studies in wild populations provide substantially lower repeatability estimates (7%, Spurgin *et al.* 2017; 13%, Fairlie *et al.* 2016). The individual

372 repeatability estimate in RLTL in our system is in the lower spectrum of qPCR-studies. Such a low
373 individual repeatability indicates that the within-individual slopes in RLTL across ages are different.
374 RLTL is therefore highly variable within individuals across their lifetimes, where positive within-
375 individual changes indicate some active process in increasing telomere length.

376 Telomere elongation, particularly in qPCR-based studies, is often attributed to measurement
377 error (Steenstrup *et al.* 2013; Verhulst *et al.* 2015). It is, however, becoming more apparent in wild
378 population studies that telomeres do elongate (Fairlie *et al.* 2016; Hoelzl *et al.* 2016a; Hoelzl *et al.*
379 2016b; Kotrschal *et al.* 2007; Spurgin *et al.* 2017). Our study supports this, using monochrome
380 multiplex qPCR that, in principle, reduces measurement error due to reactions occurring in the same
381 well. Additionally, we found that residual variance among samples was smaller than the overall change
382 in RLTL, and variance among technical replicates was smaller than among-sample variation, indicating
383 that increases in mean telomere length with age were not due to measurement error alone.

384 Aside from actual telomere elongation, however, we acknowledge the potential for competing
385 mechanisms that could alter mean RLTL, notably changes in leukocyte cell composition with age
386 (Kimura *et al.* 2010; Linton & Dorshkind 2004; Pawelec *et al.* 2010; Weng 2012). Mammalian leukocytes
387 are nucleated and different leukocyte cell types have different telomere lengths due to their respective
388 functional capacities to proliferate and express telomerase (Aubert & Lansdorp 2008; Weng 2001), and
389 these vary in ratio over time with health/immune status (see Davis *et al.* 2008). For instance, an innate
390 immune response can cause a granulocyte-biased leukocyte ratio, where in humans and baboons the
391 granulocytes have longer telomeres than lymphocytes (Baerlocher *et al.* 2007; Kimura *et al.* 2010).
392 While a previous study of RLTL in wild Soay sheep did not find changes in leukocyte cell composition
393 with age (Watson *et al.* 2017), leukocyte cell composition in badgers does vary between similar aged
394 cubs and across an individual's lifespan due to changes in immune system activation (Montes 2007). A
395 greater metabolic rate while clearing infection could also modify leukocyte cell composition and
396 potentially affect mean RLTL directly. For instance, badger cubs are typically infected with coccidia

(Newman *et al.* 2001), causing a strong innate immune response and oxidative stress (Bilham *et al.* 2018; Bilham *et al.* 2013). A change in an individual's immunological status, along with age, may therefore alter individual leukocyte cell composition and might contribute to RLTL elongation in this study.

Our study shows a positive relationship between early-life RLTL and lifespan, driven by survival benefits of long telomeres in early-life, rather than in adulthood. This is congruent with previous studies reporting that early-life RLTL predicts lifespan more strongly than RLTL in adulthood (Fairlie *et al.* 2016; Heidinger *et al.* 2012) and where early-life RLTL predicts survival to adulthood in non-human mammals (Cram *et al.* 2017; Fairlie *et al.* 2016). Early-life RLTL in badgers does predict survival to adulthood, but not adult survival probability. Cubs have higher mortality rates than adults (Macdonald *et al.* 2009), which could drive this association between early-life RLTL and lifespan. In contrast, adult RLTL in badgers did not predict survival to the following year, whereas other studies found that adult RLTL does predict survival to the next year (e.g. Barrett *et al.* 2013). The lack of such an association in our study system could be due to, for example, most of our RLTL measurements in later adulthood (≥2 years) being from long-lived individuals, indicating a sampling bias with fewer samples in later adulthood from individuals with shorter lifespans. The interplay between adult RLTL and the adult environment, or in combination with the early-life environment, also requires understanding to explain the link between adult RLTL and adult survival to the next year. Even though early-life RLTL predicts survival probability in badgers, it remains currently unclear how RLTL and life-history are linked (Simons 2015; Young 2018). A direct link might exist through delayed cellular senescence when telomeres are longer (von Zglinicki *et al.* 2001). However, an indirect link exists when telomeres function as a biomarker of somatic redundancy and reflect the accumulated damage to other biological structures that have deleterious effects on fitness (Boonekamp *et al.* 2013; Young 2018).

The early-life environment clearly exerted a strong effect on early-life RLTL, apparent from the pronounced variation in early-life RLTL we noted among cohorts, which corroborates the variation in

survival rate and lifespan among cohorts in our study system (Macdonald & Newman 2002; Macdonald *et al.* 2010). Badgers in our study are exposed to variable environmental conditions and have a limited tolerance for, for example, cohort-specific weather conditions (i.e. higher cub recruitment and survival probability with intermediate levels of rainfall and restricted deviation from the mean temperature; Nouvellet *et al.* 2013; Macdonald *et al.* 2010) and exposure to diseases (i.e. lower cub survival probability with higher intensities of coccidia; Newman *et al.* 2001). These variable environmental conditions may be reflected in the variation in early-life telomere length seen in our study system. Similarly, previous studies in birds have shown that higher levels of early-life competition can accelerate telomere shortening (Boonekamp *et al.* 2014; Nettle *et al.* 2015), although studies that do not find stressors affecting early-life telomere length do exist (reviewed in Vedder *et al.* 2017). In mammals, studies on social and ecological effects on telomere dynamics are emerging (Cram *et al.* 2017; Izzo *et al.* 2011; Lewin *et al.* 2015; Watson *et al.* 2017; Wilbourn *et al.* 2017), showing that, for example, socially dominant spotted hyaenas (*Crocuta crocuta*) have longer telomeres (Lewin *et al.* 2015) and that meerkat pups experiencing more intense early-life competition have shorter telomeres (Cram *et al.* 2017).

As well as environmental effects, variation in early-life RLTL can also be caused by additive genetic effects (Dugdale & Richardson 2018). In wild populations, using a quantitative genetic 'animal model', no heritability of telomere length was found in white-throated dippers (*Cinclus cinclus*; Becker *et al.* 2015), and high heritability (0.35 – 0.48) was found in the great reed warbler (*Acrocephalus arundinaceus*; Asghar *et al.* 2015). Even though we currently have no heritability estimates from wild mammals, the likelihood for additive genetic effects in our study system to contribute to early-life RLTL is small given that individual repeatability, which sets the upper limit for heritability (unless indirect genetic effects occur), in RLTL is low. This indicates that the individual variation in RLTL in our study system is likely driven by early-life environmental conditions.

Our findings demonstrate that telomeres reflect the effects of early-life conditions on individual life-history, and elaborate on the dynamic way that telomeres function as a biomarker of senescence in a wild mammal, where within-individual telomere length is highly variable. Further work on how specific early-life environment conditions impact telomere lengths in wild mammals and quantifying the relative contribution of environmental effects (e.g. cohort, year and social group) on telomere length will provide insight into the evolution of senescence.

Ethics

All work was approved by the University of Oxford's Animal Welfare and Ethical Review Board, ratified by the University of Leeds, and carried out under Natural England Licenses, currently 2017-27589-SCI-SCI and Home Office Licence (Animals, Scientific Procedures, Act, 1986) PPL: 30/3379.

Acknowledgements

We thank all members of the Wytham badger team, present and past, for their help in data collection and in particular Nadine Sugianto, Tanesha Allen and Julius Bright Ross. We also thank Natalie dos Remedios, Terry Burke, Mirre Simons, Simon Goodman, Keith Hamer, Elisa P. Badas and Alexandra Sparks for their help, advice and fruitful discussions on telomere analyses and comments on earlier drafts of this manuscript. We would like to thank Dan Nussey and two anonymous reviewers for comments which greatly improved the manuscript. S.H.J.v.L was funded by a Leeds Anniversary Research Scholarship from the University of Leeds with support of a Heredity Fieldwork Grant from the Genetics Society and a Priestley Centre Climate Bursary from the University of Leeds. Telomere length analyses were funded by a Natural Environment Research Council (NERC) Biomolecular Analysis Facility – Sheffield, grant to H.L.D and A.B. (NBAF984).

Authors' contributions

The study was conceived by S.H.J.v.L., A.B. and H.L.D., and developed by C.N., C.D.B. and D.W.M.; Samples were collected by S.H.J.v.L., C.N., C.D.B., D.W.M. and H.L.D.; S.H.J.v.L. conducted laboratory work and statistical analyses with input from H.L.D.; the paper was written by S.H.J.v.L. and H.L.D. and all authors critiqued the output for important intellectual content. All authors gave final approval for publication.

Data Accessibility

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.64hm348.

References

Armanios M., Blackburn E. H. (2012). The telomere syndromes. *Nature Reviews Genetics*, *13*, 693-704. https://doi.org/10.1038/nrg3246

Asghar M., Bensch S., Tarka M., Hansson B., Hasselquist D. (2015a). Maternal and genetic factors determine early life telomere length. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20142263. https://doi.org/10.1098/rspb.2014.2263

Asghar M., Hasselquist D., Hansson B., Zehtindjiev P., Westerdahl H., Bensch S. (2015b). Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science*, *347*, 436-438. https://doi.org/10.1126/science.1261121

Aubert G., Lansdorp P. M. (2008). Telomeres and aging. *Physiological Reviews*, *88*, 557-579. https://doi.org/10.1152/physrev.00026.2007

Baerlocher G. M., Mak J., Roth A., Rice K. S., Lansdorp P. M. (2003). Telomere shortening in leukocyte subpopulations from baboons. *Journal of Leukocyte Biology*, *73*, 289-296. https://doi.org/10.1189/jlb.0702361

Baerlocher G. M., Rice K., Vulto I., Lansdorp P. M. (2007). Longitudinal data on telomere length in leukocytes from newborn baboons support a marked drop in stem cell turnover around 1 year of age. *Aging Cell*, *6*, 121-123. https://doi.org/10.1111/j.1474-9726.2006.00254.x

Barrett E. L. B., Burke T. A., Hammers M., Komdeur J., Richardson D. S. (2013). Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology*, *22*, 249-259. https://doi.org/10.1111/mec.12110

Bates D., Machler M., Bolker B. M., Walker S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1-48. https://doi.org/10.18637/jss.v067.i01

Bateson M., Nettle D. (2016). The telomere lengthening conundrum - It could be biology. *Aging Cell*, *16*, 312-319. https://doi.org/10.1111/acel.12555

Bauch C., Becker P. H., Verhulst S. (2013). Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20122540. https://doi.org/10.1098/rspb.2012.2540

Beaulieu M., Reichert S., Le Maho Y., Ancel A., Criscuolo F. (2011). Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. *Functional Ecology*, *25*, 577-585. https://doi.org/10.1111/j.1365-2435.2010.01825.x

Bebbington K., Spurgin L. G., Fairfield E. A., Dugdale H. L., Komdeur J., Burke T., Richardson D. S. (2016). Telomere length reveals cumulative individual and transgenerational inbreeding effects in a passerine bird. *Molecular Ecology*, *25*, 2949-2960. https://doi.org/10.1111/mec.13670

Becker P. J. J., Reichert S., Zahn S., Hegelbach J., Massemin S., Keller L. F., . . . Criscuolo O. (2015). Mother-offspring and nest-mate resemblance but no heritability in early-life telomere length in white-throated dippers. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20142924. https://doi.org/10.1098/rspb.2014.2924

Beirne C., Delahay R., Hares M., Young A. (2014). Age-related declines and disease-associated variation in immune cell telomere length in a wild mammal. *PLoS ONE*, *9*, e108964. https://doi.org/10.1371/journal.pone.0108964

Benetos A., Kark J. D., Susser E., Kimura M., Sinnreich R., Chen W., . . . Aviv A. (2013). Tracking and fixed ranking of leukocyte telomere length across the adult life course. *Aging Cell*, *12*, 615-621. https://doi.org/10.1111/acel.12086

Bilham K., Newman C., Buesching C. D., Noonan M. J., Boyd A., Smith A. L., Macdonald D. W. (2018). Effects of weather conditions on oxidative stress, oxidative damage, and antioxidant capacity in a wild-living mammal, the European badger (*Meles meles*). *Physiological and Biochemical Zoology*, *91*, 987-1004. https://doi.org/10.1086/698609

Bilham K., Sin Y. W., Newman C., Buesching C. D., Macdonald D. W. (2013). An example of life history antecedence in the European badger (*Meles meles*): rapid development of juvenile antioxidant capacity, from plasma vitamin E analogue. *Ethology Ecology & Evolution*, *25*, 330-350. https://doi.org/10.1080/03949370.2013.767861

Blackburn E. H. (2000). Telomere states and cell fates. *Nature*, *408*, 53-56. https://doi.org/10.1038/35040500

Blackburn E. H., Greider C. W., Henderson E., Lee M. S., Shampay J., Shippenlentz D. (1989). Recognition and elongation of telomeres by telomerase. *Genome*, *31*, 553-560. https://doi.org/10.1139/g89-104

Boonekamp J. J. (2017). Does oxidative stress shorten telomeres? *Biology Letters*, *13*, 1-5. https://doi.org/10.1098/rsbl.2017.0463

Boonekamp J. J., Mulder G. A., Salomons H. M., Dijkstra C., Verhulst S. (2014). Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20133287. https://doi.org/10.1098/rspb.20133287

Boonekamp J. J., Simons M. J. P., Hemerik L., Verhulst S. (2013). Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging Cell*, *12*, 330-332. https://doi.org/10.1111/acel.12050

Buesching C. D., Newman C., Service K., Macdonald D. W., Riordan P. (2016). Latrine marking patterns of badgers (*Meles meles*) with respect to population density and range size. *Ecosphere*, *7*, e01328. https://doi.org/10.1002/ecs2.1328

Burnham K. P., Anderson D. R., Huyvaert K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*, 23-35. https://doi.org/10.1007/s00265-010-1029-6

Campbell R. D., Rosell F., Newman C., Macdonald D. W. (2017). Age-related changes in somatic condition and reproduction in the Eurasian beaver: Resource history influences onset of reproductive senescence. *PLoS ONE*, *12*, e0187484. https://doi.org/10.1371/journal.pone.0187484

Campisi J. (2005). Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. *Cell*, *120*, 513-522. https://doi.org/10.1016/j.cell.2005.02.003

Campisi J., di Fagagna F. D. (2007). Cellular senescence: when bad things happen to good cells. *Nature Reviews Molecular Cell Biology*, *8*, 729-740. https://doi.org/10.1038/nrm2233

559 Cawthon R. M. (2009). Telomere length measurement by a novel monochrome multiplex quantitative
560 PCR method. *Nucleic Acids Research*, *37*, e21. https://doi.org/10.1093/nar/gkn1027
561 Cesare A. J., Reddel R. R. (2010). Alternative lengthening of telomeres: models, mechanisms and
562 implications. *Nature Reviews Genetics*, *11*, 319-330. https://doi.org/10.1038/nrg2763
563 Cooper E. B., Kruuk L. E. B. (2018). Ageing with a silver-spoon: A meta-analysis of the effect of
564 developmental environment on senescence. *Evolution Letters*, *2*, 460-471.
565 https://doi.org/10.1002/evl3.79
566 Cram D. L., Monaghan P., Gillespie R., Clutton-Brock T. (2017). Effects of early-life competition and
567 maternal nutrition on telomere lengths in wild meerkats. *Proceedings of the Royal Society B:*
568 *Biological Sciences*, *284*, 20171383. https://doi.org/10.1098/rspb.2017.1383
569 da Silva J., Macdonald D. W. (1989). Limitations of the use of tooth wear as a means of ageing Eurasian
570 badgers, *Meles meles*. *Revue D'Ecologie La Terre et la Vie*, *44*, 275-278.
571 https://doi.org/hdl.handle.net/2042/55358
572 Davis A. K., Maney D. L., Maerz J. C. (2008). The use of leukocyte profiles to measure stress in
573 vertebrates: a review for ecologists. *Functional Ecology*, *22*, 760-772.
574 https://doi.org/10.1111/j.1365-2435.2008.01467.x
575 de Lange T. (2004). T-loops and the origin of telomeres. *Nature Reviews Molecular Cell Biology*, *5*, 323-
576 329. https://doi.org/10.1038/nrm1359
577 Delahay R. J., Brown J. A., Mallinson P. J., Spyvee P. D., Handoll D., Rogers L. M., Cheeseman C. L. (2000).
578 The use of marked bait in studies of the territorial organization of the European badger (*Meles*
579 *meles*). *Mammal Review*, *30*, 73-87. https://doi.org/10.1046/j.1365-2907.2000.00058.x
580 Dochtermann N. A., Schwab T., Sih A. (2015). The contribution of additive genetic variation to
581 personality variation: heritability of personality. *Proceedings of the Royal Society B: Biological*
582 *Sciences*, *282*, 20142201. https://doi.org/10.1098/rspb.2014.2201
583 Dugdale H. L., Macdonald D. W., Pope L. C., Burke T. (2007). Polygynandry, extra-group paternity and
584 multiple-paternity litters in European badger (*Meles meles*) social groups. *Molecular Ecology*,
585 *16*, 5294-5306. https://doi.org/10.1111/j.1365-294X.2007.03571.x
586 Dugdale H. L., Pope L. C., Newman C., Macdonald D. W., Burke T. (2011). Age-specific breeding success
587 in a wild mammalian population: selection, constraint, restraint and senescence. *Molecular*
588 *Ecology*, *20*, 3261-3274. https://doi.org/10.1111/j.1365-294X.2011.05167.x
589 Dugdale H. L., Richardson D. S. (2018). Heritability of telomere variation: it is all about the
590 environment! *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*,
591 20160450. https://doi.org/10.1098/rstb.2016.0450
592 Ellwood S. A., Newman C., Montgomery R. A., Nicosia V., Buesching C. D., Markham A., . . . Macdonald
593 D. W. (2017). An active-radio-frequency-identification system capable of identifying co-
594 locations and social-structure: Validation with a wild free-ranging animal. *Methods in Ecology*
595 *and Evolution*, *8*, 1822-1831. https://doi.org/10.1111/2041-210x.12839
596 Epel E. S., Blackburn E. H., Lin J., Dhabhar F. S., Adler N. E., Morrow J. D., Cawthon R. M. (2004).
597 Accelerated telomere shortening in response to life stress. *Proceedings of the National*
598 *Academy of Sciences of the United States of America*, *101*, 17312-17315.
599 https://doi.org/10.1073/pnas.0407162101
600 Fairlie J., Holland R., Pilkington J. G., Pemberton J. M., Harrington L., Nussey D. H. (2016). Lifelong
601 leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell*, *15*, 140-148.
602 https://doi.org/10.1111/acel.12417
603 Fell R. J., Buesching C. A., Macdonald D. W. (2006). The social integration of European badger (*Meles*
604 *meles*) cubs into their natal group. *Behaviour*, *143*, 683-700.
605 https://doi.org/10.1163/156853906777791315
606 Frenck R. W., Blackburn E. H., Shannon K. M. (1998). The rate of telomere sequence loss in human
607 leukocytes varies with age. *Proceedings of the National Academy of Sciences of the United*
608 *States of America*, *95*, 5607-5610. https://doi.org/10.1073/pnas.95.10.5607

609 Hadfield J. D. (2010). MCMC methods for multi-response generalised linear mixed models: the
610 MCMCglmm R package. *Journal of Statistical Software*, *33*, 1-22.
611 https://doi.org/10.18637/jss.v033.i02
612 Hall M. E., Nasir L., Daunt F., Gault E. A., Croxall J. P., Wanless S., Monaghan P. (2004). Telomere loss
613 in relation to age and early environment in long-lived birds. *Proceedings of the Royal Society*
614 *B: Biological Sciences*, *271*, 1571-1576. https://doi.org/10.1098/rspb.2004.2768
615 Hamilton W. D. (1966). Moulding of senescence by natural selection. *Journal of Theoretical Biology*,
616 *12*, 12-45. https://doi.org/10.1016/0022-5193(66)90184-6
617 Harrison X. A. (2014). Using observation-level random effects to model overdispersion in count data in
618 ecology and evolution. *PeerJ*, *2*, e616. https://doi.org/10.7717/peerj.616
619 Haussmann M. F., Winkler D. W., Vleck C. M. (2005). Longer telomeres associated with higher survival
620 in birds. *Biology Letters*, *1*, 212-214. https://doi.org/10.1098/rsbl.2005.0301
621 Heidinger B. J., Blount J. D., Boner W., Griffiths K., Metcalfe N. B., Monaghan P. (2012). Telomere length
622 in early life predicts lifespan. *Proceedings of the National Academy of Sciences of the United*
623 *States of America*, *109*, 1743-1748. https://doi.org/10.1073/pnas.1113306109
624 Hoelzl F., Cornils J. S., Smith S., Moodley Y., Ruf T. (2016a). Telomere dynamics in free-living edible
625 dormice (*Glis glis*): the impact of hibernation and food supply. *Journal of Experimental Biology*,
626 *219*, 2469-2474. https://doi.org/10.1242/jeb.140871
627 Hoelzl F., Smith S., Cornils J. S., Aydinonat D., Bieber C., Ruf T. (2016b). Telomeres are elongated in
628 older individuals in a hibernating rodent, the edible dormouse (*Glis glis*). *Scientific Reports*, *6*,
629 36856. https://doi.org/10.1038/srep36856
630 Izzo C., Hamer D. J., Bertozzi T., Donnellan S. C., Gillanders B. M. (2011). Telomere length and age in
631 pinnipeds: The endangered Australian sea lion as a case study. *Marine Mammal Science*, *27*,
632 841-851. https://doi.org/10.1111/j.1748-7692.2010.00450.x
633 Johnson D. D. P., Macdonald D. W., Dickman A. J. (2000). An analysis and review of models of the
634 sociobiology of the Mustelidae. *Mammal Review*, *30*, 171-196.
635 https://doi.org/10.1046/j.1365-2907.2000.00066.x
636 Jones O. R., Scheuerlein A., Salguero-Gomez R., Camarda C. G., Schaible R., Casper B. B., . . . Vaupel J.
637 W. (2014). Diversity of ageing across the tree of life. *Nature*, *505*, 169-173.
638 https://doi.org/10.1038/nature12789
639 Kimura M., Gazitt Y., Cao X. J., Zhao X. Y., Lansdorp P. M., Aviv A. (2010). Synchrony of telomere length
640 among hematopoietic cells. *Experimental Hematology*, *38*, 854-859.
641 https://doi.org/10.1016/j.exphem.2010.06.010
642 Kotrschal A., Ilmonen P., Penn D. J. (2007). Stress impacts telomere dynamics. *Biology Letters*, *3*, 128-
643 130. https://doi.org/10.1098/rsbl.2006.0594
644 Lee R. (2008). Sociality, selection, and survival: simulated evolution of mortality with intergenerational
645 transfers and food sharing. *Proceedings of the National Academy of Sciences of the United*
646 *States of America*, *105*, 7124-7128. https://doi.org/10.1073/pnas.0710234105
647 Lee R. D. (2003). Rethinking the evolutionary theory of aging: transfers, not births, shape senescence
648 in social species. *Proceedings of the National Academy of Sciences of the United States of*
649 *America*, *100*, 9637-9642. https://doi.org/10.1073/pnas.1530303100
650 Lemaitre J. F., Berger V., Bonenfant C., Douhard M., Gamelon M., Plard F., Gaillard J. M. (2015). Early-
651 late life trade-offs and the evolution of ageing in the wild. *Proceedings of the Royal Society B:*
652 *Biological Sciences*, *282*, 20150209. https://doi.org/10.1098/rspb.2015.0209
653 Lewin N., Treidel L. A., Holekamp K. E., Place N. J., Haussmann M. F. (2015). Socioecological variables
654 predict telomere length in wild spotted hyenas. *Biology Letters*, *11*, 20140991.
655 https://doi.org/10.1098/rsbl.2014.0991
656 Linton P. J., Dorshkind K. (2004). Age-related changes in lymphocyte development and function. *Nature*
657 *Immunology*, *5*, 133-139. https://doi.org/10.1038/ni1033

Macdonald D. W., Newman C. (2002). Population dynamics of badgers (*Meles meles*) in Oxfordshire, UK: Numbers, density and cohort life histories, and a possible role of climate change in population growth. *Journal of Zoology*, *256*, 121-138. https://doi.org/10.1017/S0952836902000158

Macdonald D. W., Newman C., Buesching C. D. (2015) Badgers in the rural landscape - conservation paragon or farmland pariah? Lessons from the Wytham badger project. In D. W. Macdonald, R. E. Feber (Eds.), *Wildlife conservation on farmland volume 2: Conflict in the countryside* (pp. 1-32), Oxford: Oxford University Press.

Macdonald D. W., Newman C., Buesching C. D., Johnson P. J. (2008). Male-biased movement in a high-density population of the Eurasian badger (*Meles Meles*). *Journal of Mammalogy*, *89*, 1077-1086. https://doi.org/10.1644/07-Mamm-a-185.1

Macdonald D. W., Newman C., Buesching C. D., Nouvellet P. (2010). Are badgers 'under the weather'? Direct and indirect impacts of climate variation on European badger (*Meles meles*) population dynamics. *Global Change Biology*, *16*, 2913-2922. https://doi.org/10.1111/j.1365-2486.2010.02208.x

Macdonald D. W., Newman C., Dean J., Buesching C. D., Johnson P. J. (2004). The distribution of Eurasian badger, *Meles meles*, setts in a high-density area: field observations contradict the sett dispersion hypothesis. *Oikos*, *106*, 295-307. https://doi.org/10.1111/j.0030-1299.2004.12879.x

Macdonald D. W., Newman C., Nouvellet P. M., Buesching C. D. (2009). An analysis of Eurasian badger (*Meles meles*) population dynamics: Implications for regulatory mechanisms. *Journal of Mammalogy*, *90*, 1392-1403. https://doi.org/10.1644/08-MAMM-A-356R1.1

McLaren G. W., Thornton P. D., Newman C., Buesching C. D., Baker S. E., Mathews F., Macdonald D. W. (2005). The use and assessment of ketamine-medetomidine-butorphanol combinations for field anaesthesia in wild European badgers (*Meles meles*). *Veterinary Anaesthesia and Analgesia*, *32*, 367-372. https://doi.org/10.1111/j.1467-2995.2005.00206.x

Medawar P. B. (1952) *An unsolved problem of biology.* London: H.K. Lewis.

Mendez-Bermudez A., Hidalgo-Bravo A., Cotton V. E., Gravani A., Jeyapalan J. N., Royle N. J. (2012). The roles of WRN and BLM RecQ helicases in the alternative lengthening of telomeres. *Nucleic Acids Research*, *40*, 10809-10820. https://doi.org/10.1093/nar/gks862

Monaghan P. (2010). Telomeres and life histories: The long and the short of it. *Annals of the New York Academy of Sciences*, *1206*, 130-142. https://doi.org/10.1111/j.1749-6632.2010.05705.x

Monaghan P., Haussmann M. F. (2006). Do telomere dynamics link lifestyle and lifespan? *Trends in Ecology & Evolution*, *21*, 47-53. https://doi.org/10.1016/j.tree.2005.11.007

Monaghan P., Ozanne S. E. (2018). Somatic growth and telomere dynamics in vertebrates: relationships, mechanisms and consequences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*, 20160446. https://doi.org/10.1098/rstb.2016.0446

Montes I. (2007). *Leukocyte coping capacity and leukocyte activation as a measure of stress in wild badgers*. DPhil Thesis. University of Oxford, Oxford.

Nakagawa S., Schielzeth H. (2013). A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *4*, 133-142. https://doi.org/10.1111/j.2041-210x.2012.00261.x

Nettle D., Monaghan P., Gillespie R., Brilot B., Bedford T., Bateson M. (2015). An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. *Proceedings of the Royal Society B: Biological Sciences*, *282*, 20141610. https://doi.org/10.1098/rspb.2014.1610

Newman C., Macdonald D. W., Anwar M. A. (2001). Coccidiosis in the European badger, *Meles meles* in Wytham Woods: infection and consequences for growth and survival. *Parasitology*, *123*, 133-142. https://doi.org/10.1017/S0031182001008265

Noonan M. J., Markham A., Newman C., Trigoni N., Buesching C. D., Ellwood S. A., Macdonald D. W. (2014). Climate and the individual: Inter-annual variation in the autumnal activity of the European badger (*Meles meles*). *PLoS ONE*, *9*, e83156. https://doi.org/10.1371/journal.pone.0083156

Noonan M. J., Markham A., Newman C., Trigoni N., Buesching C. D., Ellwood S. A., Macdonald D. W. (2015). A new magneto-inductive tracking technique to uncover subterranean activity: what do animals do underground? *Methods in Ecology and Evolution*, *6*, 510-520. https://doi.org/10.1111/2041-210X.12348

Nouvellet P., Newman C., Buesching C. D., Macdonald D. W. (2013). A multi-metric approach to investigate the effects of weather conditions on the demographic of a terrestrial mammal, the European badger (*Meles meles)*. *PLoS ONE*, *8*, 1-7. https://doi.org/10.1371/journal.pone.0068116

Nussey D. H., Froy H., Lemaitre J. F., Gaillard J. M., Austad S. N. (2013). Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews*, *12*, 214-225. https://doi.org/10.1016/j.arr.2012.07.004

Nussey D. H., Kruuk L. E. B., Morris A., Clements M. N., Pemberton J. M., Clutton-Brock T. H. (2009). Inter- and intrasexual variation in aging patterns across reproductive traits in a wild red deer population. *American Naturalist*, *174*, 342-357. https://doi.org/10.1086/603615

Olovnikov A. M. (1973). Theory of marginotomy - Incomplete copying of template margin in enzymic-synthesis of polynucleotides and biological significance of phenomenon. *Journal of Theoretical Biology*, *41*, 181-190. https://doi.org/10.1016/0022-5193(73)90198-7

Partridge L., Gems D. (2007). Benchmarks for ageing studies. *Nature*, *450*, 165-167. https://doi.org/10.1038/450165a

Pawelec G., Larbi A., Derhovanessian E. (2010). Senescence of the human immune system. *Journal of Comparative Pathology*, *141*, 39-44. https://doi.org/10.1016/j.jcpa.2009.09.005

Pfaffl M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*, e45. https://doi.org/10.1093/nar/29.9.e45

R Development Core Team (2019) R: a language and environment for statistical computing, R foundation for statistical computing, Vienna.

Reichert S., Stier A. (2017). Does oxidative stress shorten telomeres in vivo? A review. *Biology Letters*, *13*, 20170463. https://doi.org/10.1098/rsbl.2017.0463

Ruijter J. M., Ramakers C., Hoogaars W. M. H., Karlen Y., Bakker O., van den Hoff M. J. B., Moorman A. F. M. (2009). Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, *37*, e45. https://doi.org/10.1093/nar/gkp045

Savill P. S. (2010) *Wytham Woods: Oxford's ecological laboratory.* Oxford: Oxford University Press.

Simons M. J. P. (2015). Questioning causal involvement of telomeres in aging. *Ageing Research Reviews*, *24*, 191-196. https://doi.org/10.1016/j.arr.2015.08.002

Simons M. J. P., Stulp G., Nakagawa S. (2014). A statistical approach to distinguish telomere elongation from error in longitudinal datasets. *Biogerontology*, *15*, 99-103. https://doi.org/10.1007/s10522-013-9471-2

Spurgin L. G., Bebbington K., Fairfield E. A., Hammers M., Komdeur J., Burke T., . . . Richardson D. S. (2017). Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. *Journal of Animal Ecology*, *87*, 187-198. https://doi.org/10.1111/1365-2656.12741

Steenstrup T., Hjelmborg J. V., Kark J. D., Christensen K., Aviv A. (2013). The telomere lengthening conundrum - artifact or biology? *Nucleic Acids Research*, *41*, e131. https://doi.org/10.1093/nar/gkt370

Stoffel M. A., Nakagawa S., Schielzeth H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution*, *8*, 1639-1644. https://doi.org/10.1111/2041-210x.12797

Sudyka J., Arct A., Drobniak S., Dubiec A., Gustafsson L., Cichon M. (2014). Experimentally increased reproductive effort alters telomere length in the blue tit (*Cyanistes caeruleus*). *Journal of Evolutionary Biology*, *27*, 2258-2264. https://doi.org/10.1111/jeb.12479

van de Pol M., Verhulst S. (2006). Age-dependent traits: A new statistical model to separate within- and between-individual effects. *American Naturalist*, *167*, 766-773. https://doi.org/10.1086/503331

Vedder O., Verhulst S., Bauch C., Bouwhuis S. (2017). Telomere attrition and growth: a life-history framework and case study in common terns. *Journal of Evolutionary Biology*, *30*, 1409-1419. https://doi.org/10.1111/jeb.13119

Verhulst S., Susser E., Factor-Litvak P. R., Simons M. J., Benetos A., Steenstrup T., . . . Aviv A. (2015). Commentary: The reliability of telomere length measurements. *International Journal of Epidemiology*, *44*, 1683-1686. https://doi.org/10.1093/ije/dyv166

von Zglinicki T. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences*, *27*, 339-344. https://doi.org/10.1016/S0968-0004(02)02110-2

von Zglinicki T., Burkle A., Kirkwood T. B. L. (2001). Stress, DNA damage and ageing - an integrative approach. *Experimental Gerontology*, *36*, 1049-1062. https://doi.org/10.1016/S0531-5565(01)00111-5

Watson R. L., Bird E. J., Underwood S., Adams R. V., Fairlie J., Watt K., . . . Nussey D. H. (2017). Sex differences in leukocyte telomere length in a free-living mammal. *Molecular Ecology*, *26*, 3230-3240. https://doi.org/10.1111/mec.13992

Weng N. P. (2001). Interplay between telomere length and telomerase in human leukocyte differentiation and aging. *Journal of Leukocyte Biology*, *70*, 861-867. https://doi.org/10.1189/jlb.70.6.861

Weng N. P. (2012). Telomeres and immune competency. *Current Opinion in Immunology*, *24*, 470-475. https://doi.org/10.1016/j.coi.2012.05.001

Wilbourn R. V., Froy H., McManus M. C., Cheynel L., Gaillard J. M., Gilot-Fromont E., . . . Nussey D. H. (2017). Age-dependent associations between telomere length and environmental conditions in roe deer. *Biology Letters*, *13*, 20170434. https://doi.org/10.1098/rsbl.2017.0434

Wilbourn R. V., Moatt J. P., Froy H., Walling C. A., Nussey D. H., Boonekamp J. J. (2018). The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*, 20160447. https://doi.org/10.1098/rstb.2016.0447

Willeit P., Willeit J., Brandstatter A., Ehrlenbach S., Mayr A., Gasperi A., . . . Kiechl S. (2010). Cellular aging reflected by leukocyte telomere length predicts advanced atherosclerosis and cardiovascular disease risk. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *30*, 1649-1656. https://doi.org/10.1161/ATVBAHA.110.205492

Williams G. C. (1957). Pleiotropy, natural selection, and the evolution of senescence. *Evolution*, *11*, 398-411. https://doi.org/10.2307/2406060

Yamaguchi N., Dugdale H. L., Macdonald D. W. (2006). Female receptivity, embryonic diapause and superfoetation in the European badger (*Meles meles*): Implications for the reproductive tactics of males and females. *Quarterly Review of Biology*, *81*, 33-48. https://doi.org/10.1086/503923

Young A. J. (2018). The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*, 20160452. https://doi.org/10.1098/rstb.2016.0452

Figures & tables

Table 1: Parameter estimates from the models that best explained the relationship between telomere length and age, when accounting for selective disappearance (*n* = 1248 samples; 612 individuals). β_W = within-individual slope, β_s = selective disappearance according to age at last capture, β_B = between-individual slope, α_i = between-individual component, S.E. = standard error, d.f. = degrees of freedom. *P*-values from log-likelihood ratio tests, where significant parameters are in bold.

Parameters	**β**	**S.E.**	**d.f.**	**P-value**	**β_B (β_S + β_W)**
Model 1[†]:					
Intercept	0.6259	0.0527			
Age (≤ 29 months) (β_W)	0.000029	0.00054	1	0.958	0.000199
(>29, ≤ 65 months) (β_W)	**0.002130**	**0.00051**	**1**	**<0.001**	**0.002301**
(>65, ≤ 112 months) (β_W)	**-0.00210**	**0.00063**	**1**	**<0.001**	**-0.001924**
(> 112 months) (β_W)	**0.004008**	**0.00143**	**1**	**0.005**	**0.004179**
Sex (male)	0.008045	0.00687	1	0.242	
Cohort[§]			**23**	**<0.001**	
Lifespan (β_S)	0.000171	0.000093	1	0.068	
Model 2[†]:					
Intercept	0.6259	0.0527			
Age (≤ 29 months) (β_W)	0.000029	0.00054	1	0.958	
(>29, ≤ 65 months) (β_W)	**0.002130**	**0.00051**	**1**	**<0.001**	
(>65, ≤ 112 months) (β_W)	**-0.00210**	**0.00063**	**1**	**<0.001**	
(> 112 months) (β_W)	**0.004008**	**0.00143**	**1**	**0.005**	
Sex (male)	0.008045	0.00687	1	0.242	
Cohort[§]			**23**	**<0.001**	
α_i (β_B)	**0.004242**	**0.00138**	**1**	**0.004**	

Random effect estimates (variance): [†]Individual ID (4.851*10-5), Plate (1.067*10-3), Social group (6.062*10-5), Year (3.731*10-3), Residual (1.295*10-2); [§]Estimates ± S.E. for 24 cohorts are in the supporting information (Figure S1).

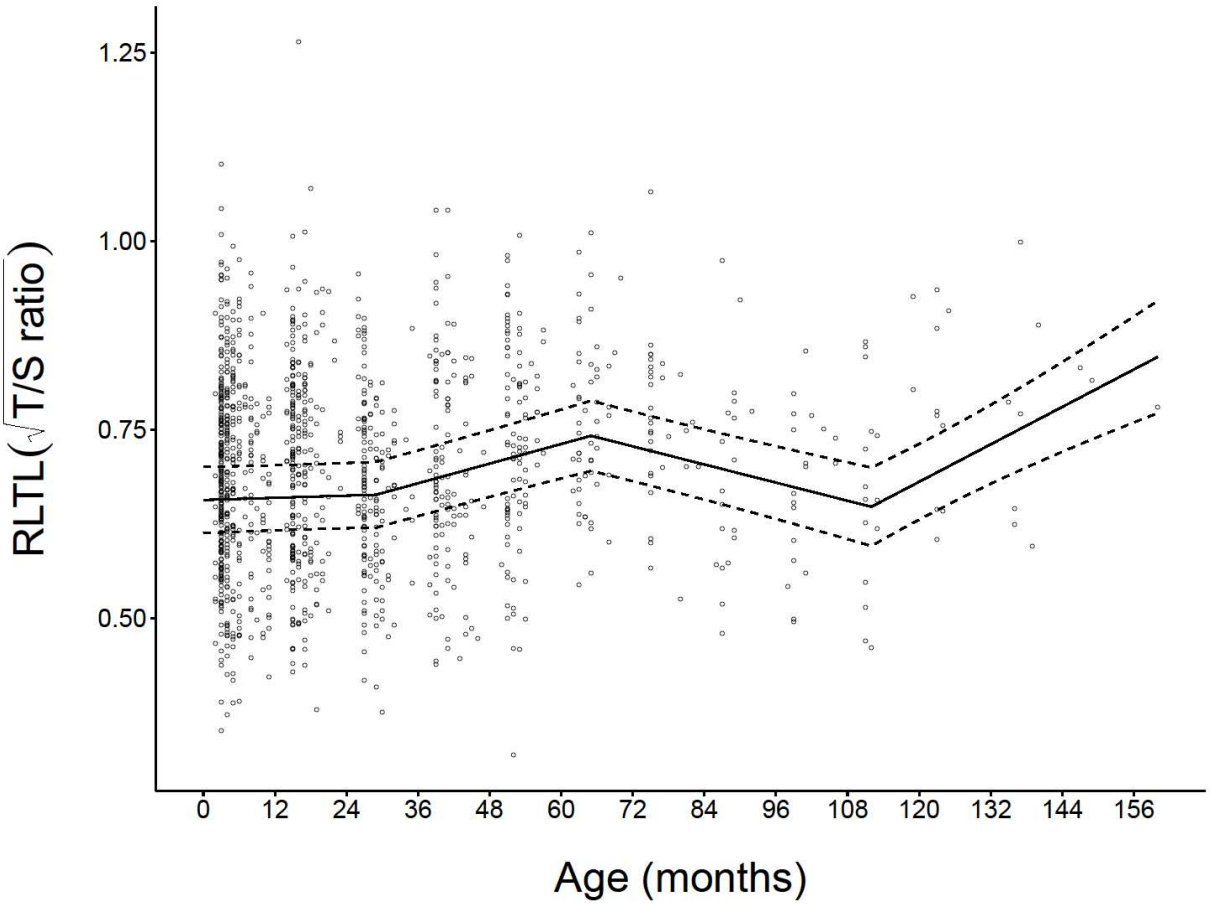

Figure 1: Age-related variation in relative leukocyte telomere length (RLTL), with inflection points at 29, 65 and 112 months of age. Raw data points (*n* = 1,248) are shown with fitted lines representing the model prediction for RLTL (T/S ratio) with 95% confidence intervals.

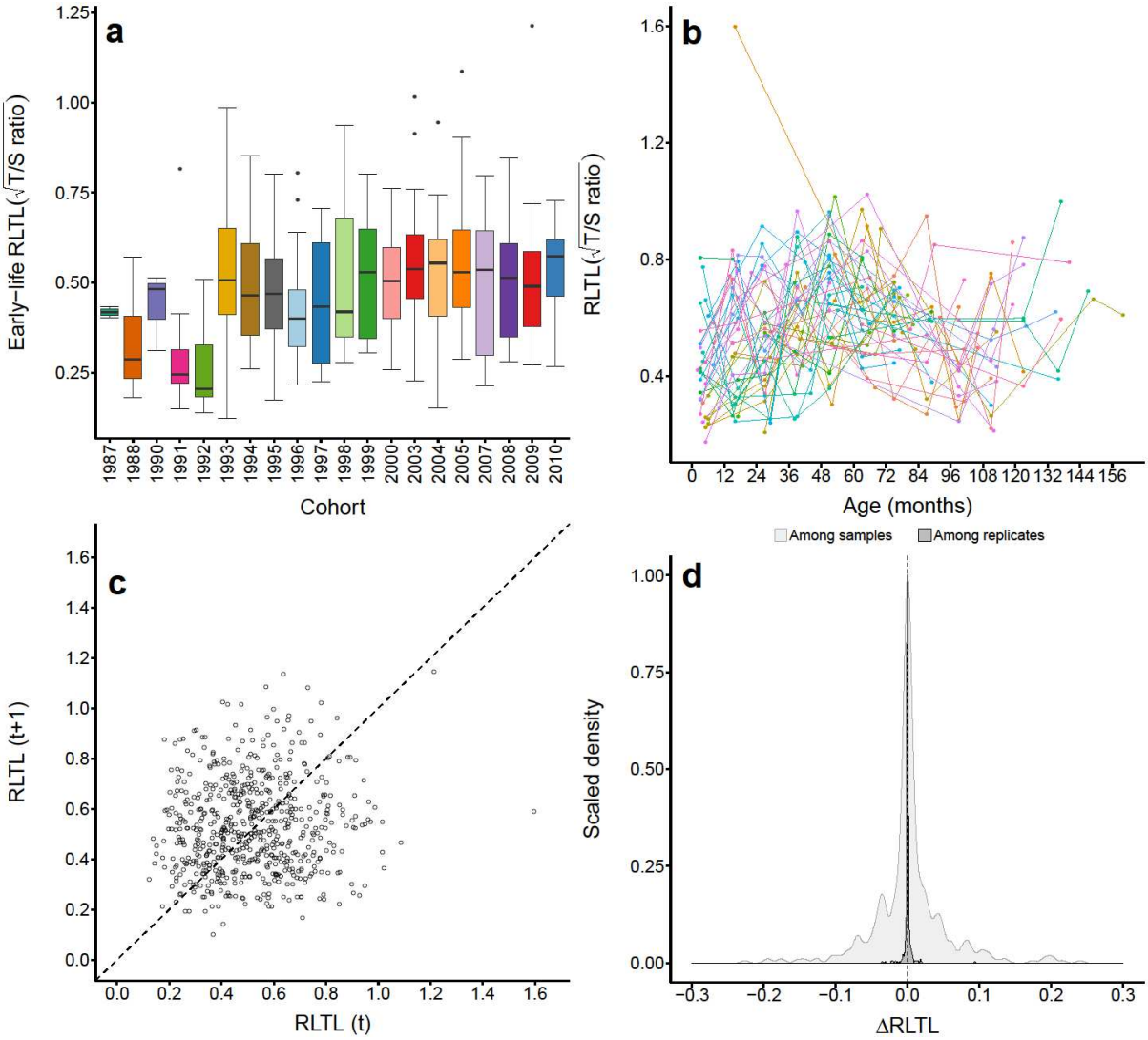

Figure 2: Telomere dynamics in European badgers. a) Variation in early-life relative leukocyte telomere length (RLTL) among cohorts. b) Longitudinal telomere dynamics for 41 individuals that were measured at least four times. c) Within-individual variation in RLTL over consecutive time points (t and t+1). Dashed line represents parity, thus data points above and below this line represent increases and decreases in telomere length, respectively. d) Scaled density plots of changes in RLTL among technical replicates (dark grey) and among individual samples (light grey) with a dotted line representing no change. Areas left of the dotted line represent decreases in RLTL, while to the right represent increases.

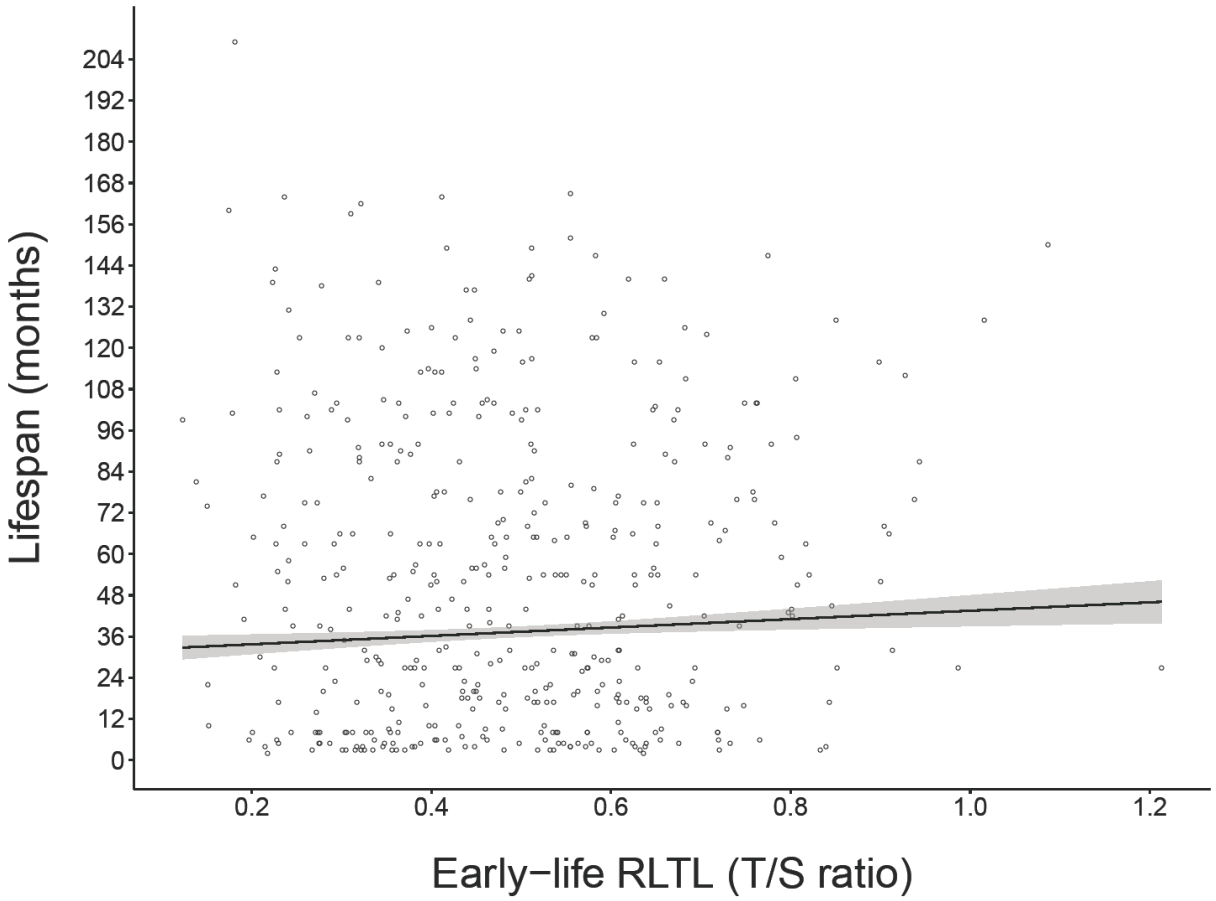

Figure 3: Early-life (<1 year old) relative leukocyte telomere length (RLTL) predicts lifespan. Raw data (*n* = 435) are shown as open circles, the regression from the GLMM as a black line, and the 95% confidence interval as the shaded area.

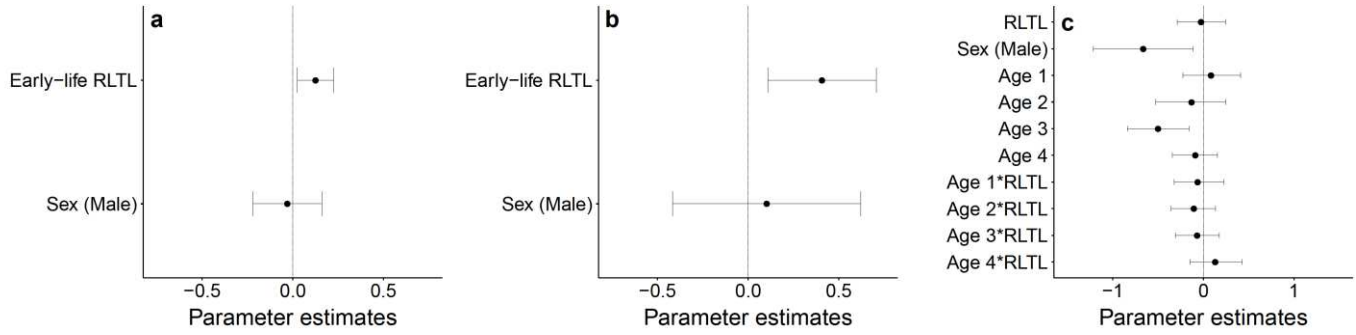

Figure 4: Parameter estimates and 95% confidence intervals of fixed effects from models investigating the effect of: a) Early-life RLTL (relative leukocyte telomere length) on lifespan; b) Early-life RLTL on survival to adulthood; and, c) Adult RLTL on survival to the next year. Age parameters in plot c) refer to threshold model where Age 1 ≤29 months old, Age 2 >29 and ≤65 months old, Age 3 >65 and ≤112 months old and Age 4 >112 months old. Scale differs in plot c). For cohort effects see Figure S2. * represents an interaction.

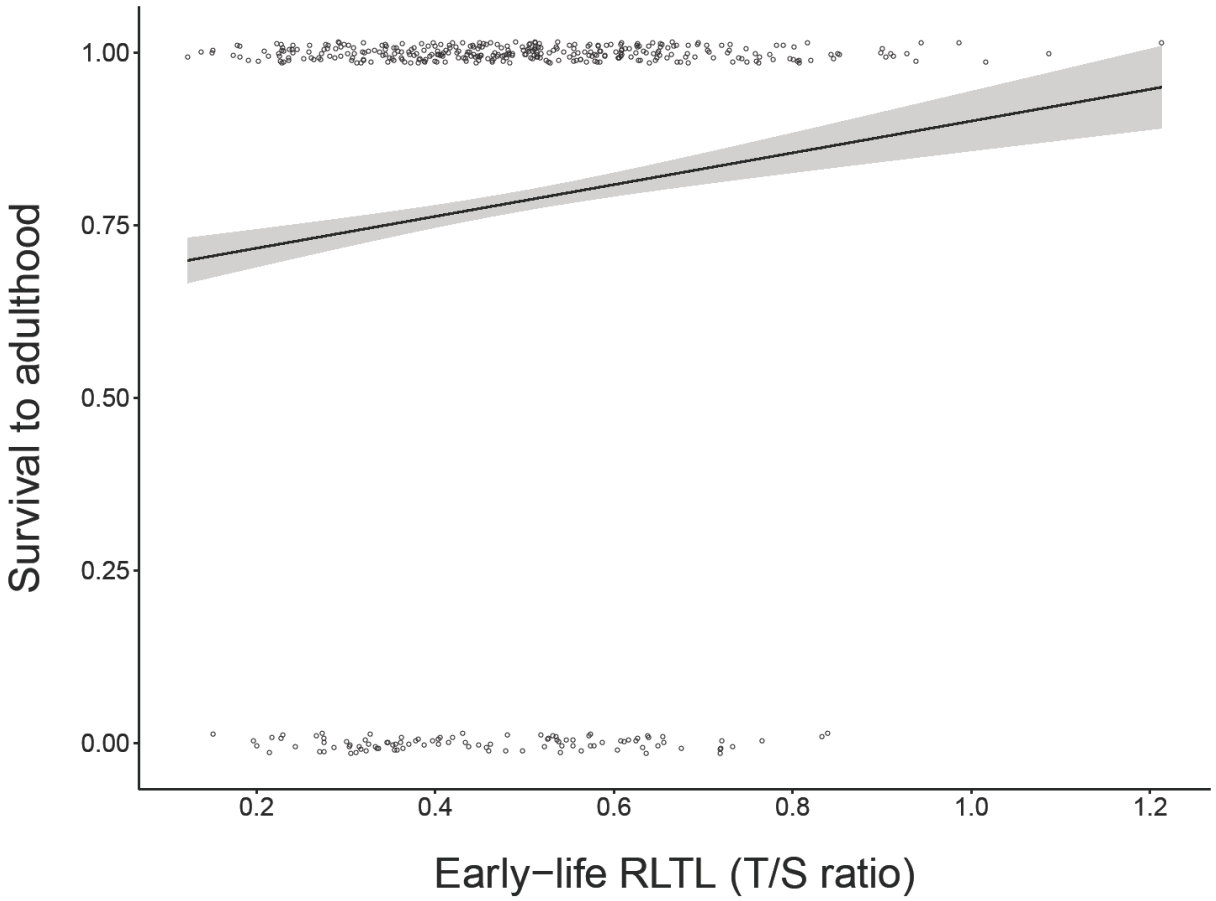

Figure 5: Early-life (<1 year old) relative leukocyte telomere length (RLTL) predicts survival to adulthood (>1 year old). The regression line from a binomial GLMM is shown, with associated 95% confidence interval as a shaded area, and raw jittered data as open circles (*n* = 435).