Telomere dynamics in wild banded mongooses: evaluating longitudinal and quasi-longitudinal markers of senescence.

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Abstract

Telomere length and the rate of telomere shortening have been suggested as particularly useful physiological biomarkers of the processes involved in senescent decline of somatic and reproductive function. However, longitudinal data on changes in telomere length across the lifespan are difficult to obtain, particularly for long-lived animals. Quasi-longitudinal studies have been proposed as a method to gain insight into telomere dynamics in long-lived species. In this method, minimally replicative cells are used as the baseline telomere length against which telomere length in highly replicative cells (which represent the current state) can be compared. Here we test the assumptions and predictions of the quasi-longitudinal approach using longitudinal telomere data in a wild cooperative mammal, the banded mongoose, *Mungos mungo*. Contrary to our prediction, telomere length (TL) was longer in leukocytes than in ear cartilage. Longitudinally, the TL of ear cartilage shortened with age, but there was no change in the TL of leukocytes, and we also observed many individuals in which TL increased rather than decreased with age. Leukocyte TL but not cartilage TL was a predictor of total lifespan, while neither predicted post-sampling survival. Our data do not support the hypothesis that cross-tissue comparison in TL can act as a quasi-longitudinal marker of senescence. Rather, our results suggest that telomere dynamics in banded

mongooses are more complex than is typically assumed, and that longitudinal studies across whole life spans are required to elucidate the link between telomere dynamics and senescence in natural populations.

1. Introduction

Senescence, the process of deterioration in somatic function with age, occurs in most organisms (Jones *et al.* 2014; Nussey *et al.* 2013) . After more than 50 years of research there is now a general consensus that senescence arises because the strength of selection on alleles with age-specific effects weakens with advancing age, and because genes with positive early life effects can be favoured even if they have deleterious effects later in life (Hamilton 1966; Kirkwood *et al.* 2000; Medawar 1952; Williams 1957). However, there is still much debate about the physiological mechanisms that lead to senescent decline in somatic and reproductive function (Briga and Verhulst 2015; Carmona and Michan 2016; Kirkwood 2011; Ljubuncic and Reznick 2009). In the last decade particular attention has focused on telomere length as a potentially important factor in the regulation of lifespan. Telomeres are repeated sequences of six nucleotides (TTAGGG) at the end sections of DNA within chromosomes. During cell division the DNA replication process leads to the loss of telomeric repeats (Aubert and Lansdorp 2008). Mechanisms do exist to restore this loss, but where these are absent or do not fully compensate the loss, repeated cell division can shorten telomeres below a critical length leading to cell death (Maser and DePinho 2004). Telomere length and shortening rate reflect damage within the organism and predict survival in the wild, and as such may be considered a 'biomarker' of cellular senescence (Monaghan 2014).

Telomere length (TL) has been found to be highly variable between individuals within the same species. In humans, various reports have suggested that shorter telomeres predispose individuals to age-related diseases such as heart disease and diabetes (Sanders and Newman 2013), and accelerated telomere loss and mutations in telomerase genes (a known mechanism for telomere length restoration) have been linked to congenital disorders and decreased life expectancy (Aubert and Lansdorp 2008). Telomere loss is also associated with negative health outcomes in non-human animals (Anchelin *et al.* 2013; Asghar *et al.* 2016; Bateson 2016; Bednarek *et al.* 2015). Much of this research takes the form of cross-sectional studies of humans and studies of laboratory model organisms. Both approaches necessarily examine populations living under artificially benign and protected conditions. A powerful complement to this research is to study the mechanistic basis of life history variation in animals exposed to natural resource limitation, predators and pathogens, where the true trade-offs involved in development and life history allocation are exposed (Hayward *et al.* 2015). In addition, to understand within-individual changes in telomere dynamics and individual heterogeneity requires longitudinal, individual-based studies. Yet, for humans and wild populations of non-human animals, longitudinal information on telomere lengths is typically lacking and difficult to obtain.

Long-term, individual-based studies of wild bird and mammal populations may offer a solution to these difficulties because individuals can be followed over the entire life course and across multiple generations in the environment in which they evolved (Clutton-Brock and Sheldon 2010). However, these studies are not without issue. First, it is often difficult to recapture the same individuals over their lifetime due to migration, dispersal and extrinsic mortality. Second, longitudinal studies require a sustained research effort over a long period of time. Many studies published to date using natural populations are based on cross-sectional data or, when longitudinal, are limited because of small sample sizes, or because information is restricted to part of the individual's lifespan. Only a few longitudinal studies have been carried out on large populations with repeated measures of individuals and accurate death dates (Barrett *et al.* 2012; Hammers *et al.* 2015; Nussey *et al.* 2013; Nussey *et al.* 2008). Longitudinal studies of cooperative mammals, in which offspring are reliant on the extended care of parents and helpers, are particularly lacking. These systems are potentially important comparators for humans, given that humans evolved in cooperatively breeding groups (Cant 2012; Clutton-Brock 2016).

Benetos et al. (Benetos *et al.* 2011) proposed a 'quasi-longitudinal' method to expand our understanding of within-individual changes in telomere length. They observed that in dogs, the length of an individual's telomeres relative to the population mean is similar in different tissues, and telomere lengths in post-mitotic tissues do not change during life. In contrast, in leukocytes, which are highly proliferative cells, telomeres were observed to shorten with age. Benetos et al. showed that, while age explained only 6% of variation in TL, it explained 43% of the variation in the *difference* between TL in post-mitotic cells such as skeletal muscle and TL in leukocytes. They proposed that this difference in telomere length between post-mitotic cells and leukocytes could be used as a quasi-longitudinal measure of within-individual changes in systems where full longitudinal sampling was not possible. Against this hypothesis, Daniali et al (Daniali *et al.* 2013) observed that in humans, age-dependent TL shortening is similar in all tissues tested. Clearly there is a need for further tests to establish the validity or otherwise of the proposed quasi-longitudinal method as a tool for evaluating within-individual telomere dynamics.

We tested the assumptions and predictions of the quasi-longitudinal method using an exceptionally well-studied wild social mammal study system, the banded mongoose *Mungos mungo*. Banded mongooses are small (ca. 1.5 kg) cooperatively breeding herpestids which live in mixed-sex groups of around 20 adults plus offspring. Since 1995 we have continuously studied a population of 200-300 animals living in Queen Elizabeth National Park, Uganda. Over 95% of the population are individually marked and regularly trapped for biometric measurements and blood sampling. For most individuals in the population we have

detailed data from birth on parentage, social care received, growth, social status, lifetime reproductive success, and survival. Unusually for mammals, in this species females have a shorter average lifespan than males (38 vs. 48 months; (Cant *et al.* 2016). This trend is also observed for maximum observed life span (females = 11 years, males = 12 years).

We used 3 years of longitudinal blood sampling from this population to test predictions of the quasi-longitudinal method that: (1) TL of leukocytes (representing highly proliferative cells) will be shorter than the TL of cartilage (representing post-mitotic, minimally proliferative somatic cells); (2) TL in proliferative cells will shorten at a higher rate than TL in somatic cells; (3) age explains variation in the difference between TL in the two tissue types. Finally, we test (4) whether TL in either tissue predicts survival in the wild and reflects the sex differences in lifespan, as expected if telomere length can be used as a biomarker of senescence.

2. METHODS

2.1.1. Study System

Data were collected from a wild population of banded mongooses living on and around Mweya Peninsula in Queen Elizabeth National Park, Uganda (0°12'S, 27°54'E). At any one time the population consists of 8-12 groups that reproduce on average four times per year, with multiple female breeders giving birth in each breeding attempt, typically on the same day (Cant *et al.* 2013). All individuals are fitted with a transponder microchip on first capture and given unique hair-shave patterns for identification. Less than half of pups survive to maturity (one year), while annual mortality for adults is around 15% (Cant *et al.* 2016; Cant *et al.* 2013) For full details of the study site, species and methods, see Cant et al. (Cant *et al.* 2013).

2.1.2. Trapping and sampling

Between 2013-2015, individuals were trapped using baited traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) and immobilised by controlled isoflurane inhalation (IsoFlo® Abbot Laboratories). Small samples of ear cartilage clippings were taken and stored in 10x volume 96% ethanol. Blood samples (taken every ~9.5 months) (volume 100-500µl) were drawn from the jugular vein within 5 min following anaesthesia, using a 25G needle and syringe and immediately transferred into a 3 ml EDTA BD Vacutainer®. Leukocytes were

separated from whole blood using micro haematocrit capillaries without heparin (VWR International) and centrifuged at 13,000g for 4 mins. The resultant buffy coat layer was collected and snap frozen using liquid nitrogen and stored at -80°C until DNA extraction.

2.1.3. Ethics and Permits

Research was conducted under permit issued from the Uganda Wildlife Authority and the Uganda National Council for Science and Technology. This work has been approved by the University of Exeter Ethics Committee. All work adhered to the Guidelines for the Treatment of Animals in Behavioural Research and Teaching, published by the Association for the Study of Animal Behaviour.

2.2.1. DNA extraction

Ear clippings were removed from ethanol and air dried. Fur, skin and any other matter was removed using a scalpel. Each sample was placed in a 1.5ml tube and frozen in liquid nitrogen, then crushed using a micropestle. DNA was extracted using DNeasy blood and tissue extraction kit (Qiagen) according to the manufacturer's instructions. DNA was eluted in 100µl TE buffer and stored at -20°C until qPCR analysis. Leukocytes were gently thawed on ice and DNA was extracted using GeneJet whole blood DNA extraction kit (Fermentas) according to the manufacturer's instructions. DNA was eluted in 100µl TE buffer and stored at -20°C until qPCR analysis. Before qPCR analysis all DNA samples were treated with RNAse A (10mg/ml) for 4 mins at room temperature. Concentration and purity was determined using Nanovue spectrometer (GE Healthcare) and Qubit Fluorometric quantitation (ThermoFisher Scientific) and DNA degradation was assessed by 2% gel electrophoresis.

2.2.2. Estimation of telomere length by Quantitative real-time PCR (qPCR)

Relative TL was determined as the ratio of telomere repeat copy number compared to the non-variable control gene (NVCG) standardised to a common (golden) sample run in parallel. qPCR reactions were carried out as previously described (Cawthon 2009) with minor amendments. For telomere PCR, 20ng of DNA was added to Brilliant II SYBR® Green Low ROX QPCR Master Mix (Agilent) with 100nM Tel1bF and 400nM Tel2bR respectively in a total reaction volume of 25µl. qPCR was a two-step reaction profile at 95°C for 10 min,

followed by 30 cycles of 95°C for 30s, 58°C for 60 s, followed by a melt curve and at 95°C for 30 s, 55°C for 30 s, 95°C for 30 s using an Mx3000P qPCR system (Agilent). For NVCG PCR, 300mM of forward and reverse primers directed towards *Mungos mungo* inter-photoreceptor retinoid-binding protein (IRBP) gene (Accession number AY170065) were used (IRBPF 5' ACC TGC ACC CAG GGA ACA CAG T 3' and IRBPR 5' GGC AGG GTC CAG ATC TCA GTG GT 3'). The qPCR reaction profile was 95°C for 10 mins followed by 40 cycles of 62°C for 30s, and 95°C for 30s followed by a melt curve and 95°C for 30s, 55°C for 30s, 95°C for 30s. All samples were run in triplicate. The Cq values for the three samples were averaged and standard error calculated. Samples with average Cq values with a standard error above 1.5 were discarded. A standard curve was run on all plates comprising 1:2 serial dilutions of a pool of DNA (resulting in total DNA concentrations of 40, 20, 10, 5 and 2.5ng) along with a no template control and 'golden' sample to which all other samples were compared.

2.2.3. qPCR Analysis

All samples were analysed as previously described by Beirne et al (Beirne *et al.* 2014). Briefly, LinRegPCR (v2013.0) (Ruijter *et al.* 2009) was used to correct for baseline fluorescence and determine the window of linearity per amplicon and threshold values determined (Cq). Cq values were plotted against log DNA concentration in order to determine the amplification efficiency of both Telomere and IRBP PCR reactions for each plate. Those falling outside 95%-110% were repeated according to MIQE guidelines (Bustin *et al.* 2009). To reconcile differences in amplification efficiency between plate runs we used the following equation;

$$X_0 = 10[(Cq-b)/m]$$

where X_0 = initial starting quantities, Cq = cycle at which the focal sample crosses the threshold (Nq), b = plate specific intercept of the log of the standard curve, and m = plate specific slope of the log of the standard curve. The amount of telomere in the focal sample was then normalised to the initial quantity of DNA in the sample by using the formula;

$$X_0 \text{ sample} = X_0 \text{ TEL} / X_0 \text{ IRBP}$$

Finally, relative telomere length (RTL) was calculated by normalising the focal sample to the golden sample;

$$\text{RTL} = X_0 \text{ sample} / X_0 \text{ golden sample}$$

Across all plates, the mean standard curve amplification efficiencies were 102.6% (SE ± 1.9) for telomere and 106.8% (SE ± 2.7) for the IRBP. The R^2 for each standard curve was >0.99.

Amplicon specific within-plate variability was determined by examining the standard deviation of the triplicate Cq values for each sample across each plate. The median and inter-quartile range of the standard deviations across all plates was 0.094 (0.001-0.135) for telomere and 0.073 (0.0004-0.132) for IRBP. In order to determine between-plate repeatability, 24 randomly selected samples (a single plate) were each run three times (each run once for telomere and once for IRBP, totalling 6 plates). The coefficient of variation in the relative telomere length estimates across all samples was 11.5%, and plate number was included as a random factor in all statistical analyses (see below).

2.4. Statistical Analysis

Overall we had 228 samples available from 107 individuals of known age. For the survival analyses, individuals were assigned as dead if their exact date of death was known, and censored if the date was unknown (e.g. due to dispersal out of the study population, N=3) or the individual still alive at the end of the sample collection period (1.1.2015; N = 39).

We used linear models (LMM) to investigate differences in TL between the tissue types (prediction 1), and sex and age differences in cartilage and leukocyte TL (Prediction 2). All model residuals were visually investigated for normality, homogenous variance and influential observations. To account for multiple samples per individual and possible genetic differences among family groups, we included individual and social group as random effects in the models. PCR plate was also included as a random effect to account for between-plate variation. The significance of terms was determined using likelihood ratio tests (Bates *et al.* 2015). Non-significant interactions were dropped from final models to allow significance testing of the main terms (Engqvist 2005) , but we did not reduce the models further, and report parameter estimates from the full models. All analyses were done in R (R Core Development Team 2016) using package lme4 (Bates *et al.* 2015).

Standard output from linear mixed models does not allow for distinguishing between within- and between-subject effects, such as individual reaction norms/quality and population level effects such as selective disappearance or cohort effects (Nussey D. H. *et al.* 2009; van de Pol and Verhulst 2006; van de Pol and Wright 2009). Therefore we used within-subject centering as described in van de Pol & Wright (van de Pol and Wright 2009) to examine the role of within vs. between-individual variance in describing the population level changes in

telomere length. Briefly, this involves constructing two mixed effects models. In model 1, individual mean sampling age, and within-individual deviation from the mean sampling age, are both used as predictors of telomere length, the former describing the between-individual component of change in TL, and latter describing the within-individual component. In model 2, age at sampling and individual mean age at sampling are used as predictors, age at sampling in this case being identical to the within-individual component of model 1, and mean age representing the difference between the within- and between-individual effects. Both these models were constructed separately to predict leukocyte and cartilage TL.

To test prediction 3, for 11 individuals from which we had assessed telomere length in cartilage and leukocytes in the same trapping event, difference between leukocyte TL and cartilage TL was calculated. The difference was used as the response variable in a simple linear model, with age as a predictor, to test the prediction of the quasi-longitudinal approach that the *difference* in TL between the tissue types decreases with age*;* sample size was too low to include random effects in this model, so only age at sampling was included as a predictor in this model.

Finally, we used a survival analysis to test prediction 4, that telomere length predicts post-sampling survival and is associated with sex differences in total lifespan, with sex, telomere length and their interaction as explaining factors in Cox regression models, as implemented in SPSS 23.0.0 (IBM Statistics).

3. RESULTS

3.1. Is telomere length in leukocytes shorter than in cartilage cells?

Leukocyte telomeres were consistently longer than ear cartilage telomeres(tissue type: $\beta \pm$ SE = 0.87 ± 0.21, $\chi^2_1 = 44.68$, $p < 0.00$, Fig. 1A). Although males live longer than females in this species (Vitikainen et al 2016), there were no sex differences in telomere length in ear cartilage ($\beta \pm$ SE = -0.15 ± 0.14, $\chi^2_1 = 1.11$, $p = 0.292$) nor in leukocytes ($\beta \pm$ SE = 0.14 ± 0.16, $\chi^2_1 = 0.67$, $p = 0.414$; Fig. 1A).

3.2. Does TL in leukocytes shorten at a faster rate than in cartilage cells?

Overall, age had no effect on TL in either tissue (ear cartilage: $\beta \pm$ SE = 0.002 ± 0.03, $\chi^2_1 = 0.01$, $p = 0.962$, N = 105 samples, 79 individuals); leukocytes: $\beta \pm$ SE = 0.0001 ± 0.001, χ^2_1

= 0.01, p = 0.912, N = 116 samples, 64 individuals; Fig. 1B). To determine if this was due to cross-sectional sampling bias we tested for age effects separately excluding the individuals that only had been sampled once, and the results were qualitatively the same, with no significant effects of age on TL in either tissue (ear cartilage: $\beta \pm SE = -0.032 \pm 0.06$, $\chi^2_1 = 0.19$, $p = 0.66$, N= 25 samples from 13 individuals; leukocytes: $\beta \pm SE = 0.11 \pm 0.086$, $\chi^2_1 = 1.75$, $p = 0.186$, N = 78 samples, 27 individuals).

Splitting the variation into within- and between-individual components in the longitudinal dataset, there was no change with age in leukocyte TL in either (between-individual: $\beta \pm SE = 0.0001 \pm 0.0002$, $\chi^2_1 = 0.60$, $p = 0.437$, within-individual: $\beta \pm SE = 0.0004 \pm 0.0004$, $\chi^2_1 = 0.85$, $p = 0.358$). In ear cartilage, there was a significant within-individual decline in TL ($\beta \pm SE = -0.0009 \pm 0.0004$, $\chi^2_1 = 4.02$, $p = 0.045$, Fig. 1C) but no decline in the between-individual component ($\beta \pm SE = -0.00004 \pm 0.001$, $\chi^2_1 = 0.076$, $p = 0.783$), and the difference between the two slopes was non-significant ($\beta \pm SE = 0.0008 \pm 0.0005$, $\chi^2_1 = 4.01$, $p = 0.066$) which may reflect low power of the analysis (N = 25 samples from 13 individuals). We did however also observe lengthening of TL in ear cartilage tissue in 5 out of 13 individuals, and in leukocytes in 13 out of 27 individuals (see supplementary Figure S2, which corroborates the lack of overall pattern with age).

3.3. Does age explain the difference in TL between tissue types?

Contrary our expectation, the difference in TL between tissues did not decrease with age ($\beta \pm SE = 0.0007 \pm 0.0003$, $\chi^2_1 = 0.98$, $p = 0.06$; Fig. S3).

3.4. Does telomere length in either tissue predict survival?

Neither leukocyte nor cartilage TL predicted post-sampling survival (Cox regression coefficient ± SE: Ear cartilage: $\beta = 0.202 \pm 0.166$, $p = 0.224$, N = 99, leukocytes: $\beta = 0.111 \pm 0.281$, $p = 0.693$, N = 65). Longer leukocyte TL predicted longer total lifespan (Cox regression: $\beta = -0.741 \pm 0.362$, $p = 0.041$, N = 65); the effect seemed to be driven by TL predicting survival in males but not in females, however the difference was not statistically significant (sex * TL: $\beta = 0.852 \pm 0.484$, $p = 0.078$, Fig. 1D). Cartilage TL did not predict total lifespan in either sex ($\beta = 0.225 \pm 0.165$, $p = 0.172$, N = 99).

4. DISCUSSION

4.1 Telomere length in cartilage is shorter than in leukocytes

We found that telomere length was consistently longer in leukocytes than in post-mitotic cartilage cells (Fig. 1A). This contrasts with previous studies of humans, non-human primates and dogs, all of which have found TL in leukocytes to be shorter than in minimally-proliferative cells (Benetos *et al.* 2011; Daniali *et al.* 2013). The proposed explanation for these previous findings is that blood cells reflect the telomere length of a continuously dividing haematopoetic stem cell population, in which telomeres are constantly eroded (Benetos *et al.* 2011; Daniali *et al.* 2013). Somatic cells, by contrast, are post-mitotic or have low replicative rates, and so (it is hypothesised) have less eroded telomeres. Our finding suggests that this hypothesis does not apply to all blood-versus-soma tissue comparisons, and that processes underlying tissue differences in telomere length may be more complex than assumed by the hypothesis. Indeed, other recent evidence suggests there is substantial variation in TL within and between tissues, and that TL is not necessarily inversely related to tissue replication rate. Lin et al. (2016) show that attrition rates vary between subpopulations of leukocytes in humans, which suggests average leukocyte TL may also vary according to changes in the composition of the leukocyte population being measured. In captive zebra finches, TL in blood erythrocytes (RBC) is longer than in other tested cell types, with the exception of pectoral muscle, although the difference was only statistically significant between RBC and spleen (Reichert *et al.* 2013).

There are also theoretical grounds to question the expectation that TL should be shorter in highly replicative tissues. If the stem cells of particular tissues are destined to undergo multiple rounds of mitotic division across the lifespan, selection should favour mechanisms to delay or reverse the process of telomeric attrition that leads to cell death and loss of tissue function. This argument is a version of the ‘Henry Ford principle’ of senescence, which suggests that selection will act to equalize the mortality risks accruing from damage to each tissue or system of the organism’s body (Dawkins 1995; Humphrey 1984; Laird and Sherratt 2009). In terms of TL, the mortality risks due to telomere erosion in any particular tissue depend on the initial telomere length of tissue-specific stem cells; the replication rate of those stem cells; and the degree to which telomerase is used to restore telomere length. There is little evidence that haemopoietic cells have a large initial TL to offset their higher replicative rate: in foetuses there is evidence that TL is equal across different tissues (Benetos *et al.* 2011; Daniali *et al.* 2013). However, it is possible that telomerase plays a key, and still dimly understood, role influencing the telomere lengths of rapidly dividing cell populations. Preliminary studies in banded mongooses have shown that telomerase is

expressed in leukocyte populations in this system (M. Hares unpublished). In our longitudinal dataset, many individuals appeared to show an increase in TL (Figure S2); moreover, other recent longitudinal studies of wild animals have reported similar within-individual increases in TL over time (Fairlie *et al.* 2016); Richardson et al unpublished data). In humans, telomerase is expressed during spermatogenesis, and continuously dividing sperm cells exhibit longer telomeres than somatic cells (Aston *et al.* 2012). This all suggests that telomerase activity is a possible, to date untested, explanation for lack of decline in leukocyte TL in our study.

4.2. Telomere length as a marker of senescence

Across the population, TL did not decline with age in either cartilage or leukocyte cells (Fig 1B). Longitudinally, however, there was a significant within-individual decline in TL in cartilage tissue, but not in leukocytes. These results suggest that cross-sectional patterns of telomere length in this population are largely driven by between-individual effects, such as variation in quality that may cause the selective disappearance of individuals with high rates of attrition. While telomeres in cartilage tissue showed the expected pattern of shortening with age, telomeres in highly replicative leukocytes do not show a simple age-related decline in this system.

If telomeres shorten with mitotic cell division, why did we not find the predicted pattern of shortening TL with age? There are several possible explanations. First, there may be an underlying within-individual decline with age that we are unable to detect because of lack of statistical power. In this species there is much between-individual variation in life history trajectories, which may mask the effects of chronological age. For example, males show consistent lifetime differences in the amount of cooperative and reproductive behaviour they exhibit (Sanderson *et al.* 2015), whereas the majority of females conceive at each breeding attempt, allosuckling pups born in the synchronous litters (Cant *et al.* 2016). Interestingly, the predictive effect of TL on lifespan seemed to be stronger in males than in females, which could reflect the relatively higher variance in timing of reproduction in males, as compared to females; most females breed regularly once they are one year old, while males typically do not achieve paternity until 3 or 4 years old (Cant *et al.* 2016). Future analyses of within-individual changes in telomere length should therefore be conducted in a larger dataset, where controlling for an individual's reproductive and helping history is statistically feasible. A second explanation is that there is a decline in TL across the lifespan, but that this is driven by periods of rapid decline that occur outside of the time window that we have sampled. For example, studies of wild European starlings (*Sturnus vulgaris*), barnacle geese (*Branta leucopsis*), great frigatebirds (Fregata minor), humans (*Homo sapiens*) and Soay sheep (*Ovis aries*) have all

shown greatest TL attrition to occur from birth to sexual maturity (Anchelin *et al.* 2011; Benetos *et al.* 2011; Berglund *et al.* 2016; Fairlie *et al.* 2016; Heidinger *et al.* 2012; Juola *et al.* 2006; Nettle *et al.* 2013; Parolini *et al.* 2015). Upon sexual maturity, the rate of TL attrition levels off and TL may even increase with age until very late in life, when an accelerated decline in TL is observed (Berglund *et al.* 2016; Fairlie *et al.* 2016; Hammers *et al.* 2015). Only 16 of 110 individual samples from our study were less than one year old and we have very few samples from very old individuals in the population. Deviations from a linear decline in TL with age, and in particular the potential for telomere length to increase with age in some tissues, suggests a role for telomerase in mediating telomere attrition across the lifespan. Finally there is increasing evidence to suggest that the environment conditions can affect TL and the rate of telomere attrition (Fairlie *et al.* 2016; Hammers *et al.* 2015; Mizutani *et al.* 2013; Monaghan 2014; Rollings *et al.* 2014). For example, Mizuatani et al. 2013, observed in black tailed gulls neither sex nor age predicted TL, but that increases and decreases of TL within individuals were associated with favourable or adverse conditions. This suggests that controlling for environmental conditions, as well as individual vulnerability to them will be required to see if the patterns presented herein are maintained through an individual's life-time.

4.3. Telomere length and survival

Leukocyte TL predicted total lifespan, but not post-sampling survival (Fig. 1D). These results provide support for the hypothesis that TL in this tissue is a marker of overall somatic state or functional integrity contributing to survival. According to this hypothesis, TL should be positively correlated with total lifespan. TL and post-sampling survival, however, are not expected to correlate because (i) leukocyte TL does not vary with age (Fig. 1C)(across the period sampled) and (ii) there is no detectable actuarial senescence in our population, i.e. age-specific mortality rate does not increase with age (Vitikainen et al. unpublished data). Several other studies of wild mammals have found that TL predicts total lifespan (Anchelin *et al.* 2011; Benetos *et al.* 2011; Hammers *et al.* 2015; Heidinger *et al.* 2012). While a number of studies across a range of taxa have shown a decrease in TL, or highest rates of telomere loss with increasing age (Aubert and Lansdorp 2008; Beirne *et al.* 2014; Brummendorf *et al.* 2002; Hall *et al.* 2004; Pauliny *et al.* 2012; Zeichner *et al.* 1999) several recent studies have observed no change in overall TL with age similar to our data (Fairlie *et al.* 2016; Hammers *et al.* 2015; Haussmann *et al.* 2007) or even a positive relationship between TL and age (Vleck *et al.* 2003).

Since males live longer than females in banded mongooses, we tentatively predicted that males would exhibit longer TL than females. Contrary to this prediction, we found no difference in TL between males and females (Fig. 1A), suggesting that sex-differences in lifespan are not reflected in sex-differences in the TL of either leukocytes or cartilage. This could be because differences in lifespan between males and females are relatively small in this species: the median lifespan of individuals that survive to 1 year is 42 months for males compared to 38 months for females. These differences in survival may be insufficient to shape sex-specific telomere length, or there may be small sex differences which our sample size is insufficient to detect. Additionally, if the overall difference in male and female lifespan is driven by life-history variation between the sexes, unaccounted-for within-sex variation in traits such as reproductive history, as discussed above, could be masking the effect of sex in our sample. The non-significant trend of the link between survival and leukocyte telomere length being driven by males, which are the more variable sex in terms of timing of reproduction, suggests this may be the case. Studies in other species have also found mixed evidence, with longer telomere lengths reported in the longer-lived sex in some (Gopalakrishnan *et al.* 2013; Kimura *et al.* 2008; Monaghan 2010; Njajou *et al.* 2009; Olsson *et al.* 2011; Young *et al.* 2013) but not in other studies (Heidinger *et al.* 2012; Pauliny *et al.* 2012; Turbill *et al.* 2012). The links between sex differences in telomere dynamics and lifespan remain poorly understood.

In conclusion, we found no support for the hypothesis that comparison of TL in proliferative and somatic cells offers a quasi-longitudinal measure of within-individual telomere dynamics. Telomere dynamics in different tissue types in this wild mammal system appear to be complex and likely influenced by telomerase activity. However, our results do provide some evidence in support of the hypothesis that TL in some tissues may be a useful marker for overall somatic quality in wild animal populations.

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7. Author contributions

Study was conceived by EV, MC and MH, and analyses designed by EV, MC and JB. EV, FT and HM collected the data, and MH conducted the laboratory analyses. Statistical analyses were done by EV, HM and MH, and the manuscript was drafted by MH, EV and MC, with input from all authors.

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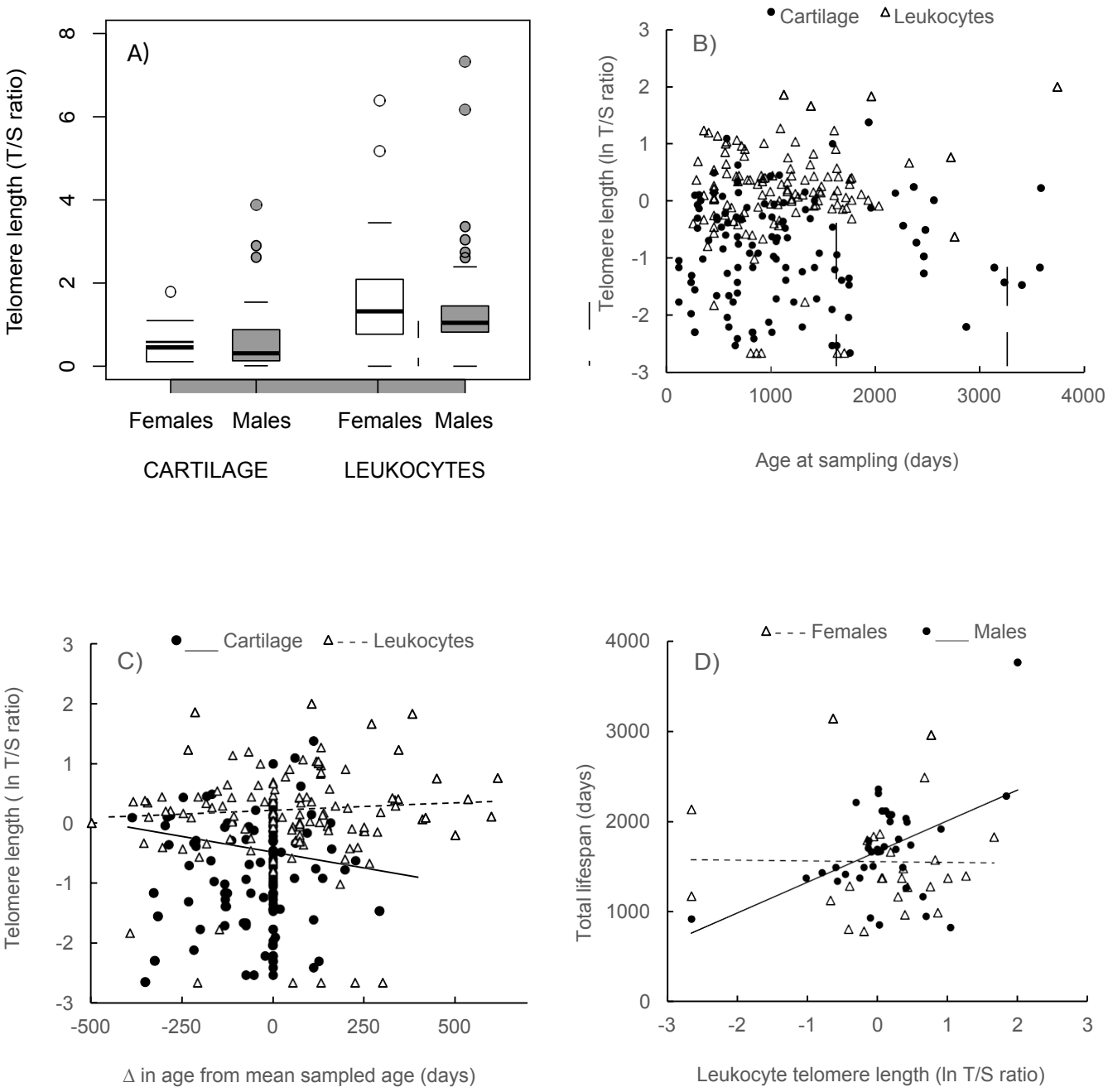


Figure 1.

A) Leukocyte telomeres were consistently longer than cartilage tissue telomeres, and there was no sex difference in either. B) There was no cross-sectional decline in telomere length with age, neither in cartilage tissue (black circles) nor leukocytes (open triangles). C) The slope for within-individual change in TL was significant for cartilage (black circles) but not for leukocytes (open triangles).

D) Leukocyte telomere length was predictive of total lifespan, and the effect appears to be driven by a positive trend in males (black circles, solid line) and not in females (open triangles, dotted line) although the sex difference was not statistically significant ($p = 0.078$); see text for details.

Fitted lines are predicted slopes from linear mixed models on ln-transformed data. Graphs and models included data from individuals that were sampled on 1–6 occasions except for D, which only includes last sampling point for each individual. In 1C points with a mean age of 0 years were sampled once.

SUPPLEMENTARY MATERIAL FOR MANUSCRIPT

Supplementary Figures

Fig. S1:

Absolute telomere length estimation by Terminal Restriction Fragment Method (TRF). DNA was extracted and analysed by TRF as previously described by Haussmann using restriction enzymes HinfI, Hae III and RSA (Haussmann and others 2005). TRF shows that the telomere length ranges from 12.9kb to 15.5kb. No interstitial repeats were observed in the *Mungos mungo* samples tested.

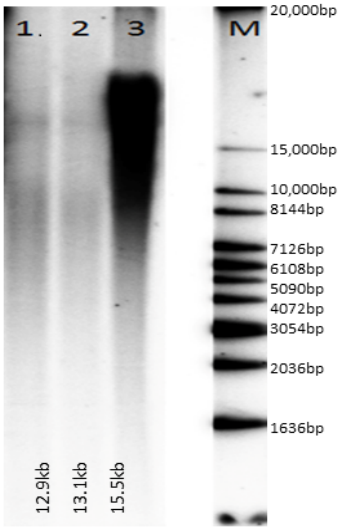


Fig. S2

Change in TL in cartilage (black circles) and in leukocytes (open triangles) with change in age. Each symbol corresponds to the observed change within individual between the sampling points. Although telomeres appeared to shorten in most cases (points below the horizontal line) there were also cases where they appeared to lengthen (points above the line).

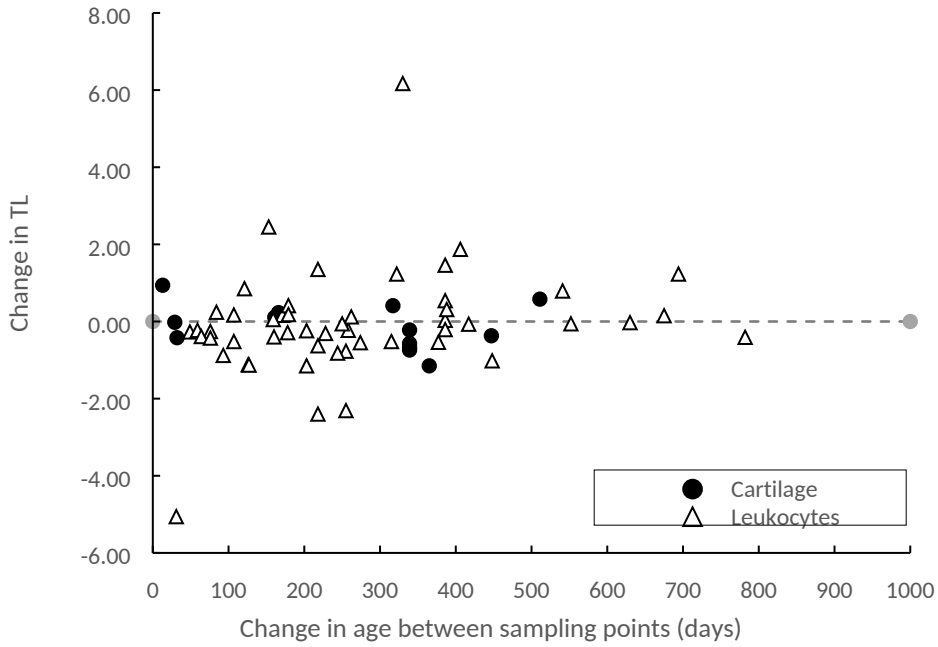
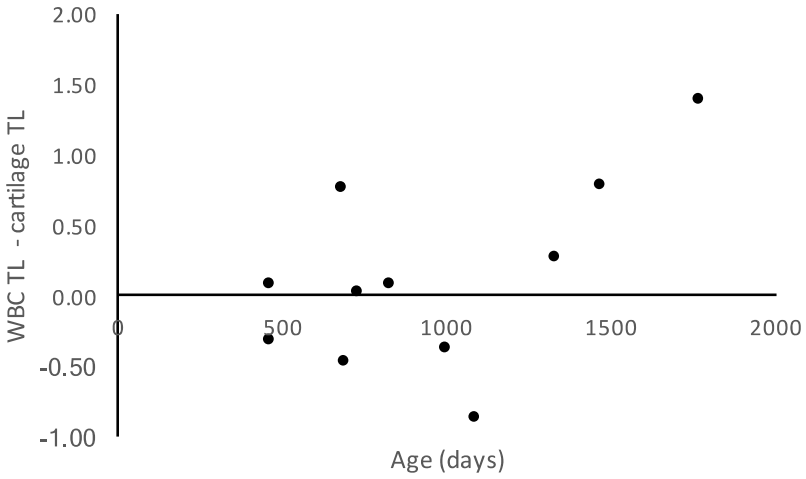


Fig. S3

Contrary to the expectation of the quasi-longitudinal approach (Benetos et al. 2011), there was a near-significant increase in the difference in TL between ear cartilage and leukocytes from the same sampling point with age.



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