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Transmissible cancer and longitudinal telomere dynamics in Tasmanian devils (*Sarcophilus harrisii*)

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Abstract

A plethora of intrinsic and environmental factors have been shown to influence the length of telomeres, the protector of chromosome ends. Despite the growing interest in infection–telomere interactions, there is very limited knowledge on how transmissible cancers influence telomere maintenance. An emblematic example of transmissible cancer occurs in the Tasmanian devil (*Sarcophilus harrisii*), whose populations have been dramatically reduced by infectious cancer cells. To investigate associations between telomere dynamics and the transmissible cancer, we used longitudinal data from a Tasmanian devil population that has been exposed to the disease for over 15 years. We detected substantial temporal variation in individual telomere length (TL), and a positive significant association between TL and age, as well as a marginally significant trend for devils with devil facial tumour disease (DFTD) having longer telomeres. A proportional hazard analysis yielded no significant effect of TL on the development of DFTD. Like previous studies, we show the complexity that TL dynamics may exhibit across the lifetime of organisms. Our work highlights the importance of long-term longitudinal sampling for understanding the effects of wildlife diseases on TL.

KEYWORDS

age, longitudinal telomere dynamics, parasites, sex, Tasmanian devil, transmissible cancer

1 | INTRODUCTION

Telomeres consist of tandem repeats of (TTAGGG) n complexes and telomere-associated proteins located at the terminal ends of

eukaryotic chromosomes (Blackburn, 1991). Their primary functions are to ensure correct chromosome segregation during mitosis, and to prevent chromosome fusion and concomitant cell-cycle arrest, caused by the terminal ends of chromosomes being treated

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as DNA double-strand breaks (Harley et al., 1990). Suppression of telomerase, the enzyme responsible for replenishing terminal telomere copies, and concomitant telomere attrition in somatic cells, present as cell-proliferation barriers and thus as repressors of malignant cancer cells (i.e., the Hayflick limit; Hayflick & Moorhead, 1961; Shay & Wright, 2000). Inhibiting tumour formation via limiting cell proliferation has the side-effect of causing replicative senescence and the progressive degradation of tissue function with advancing age (Weinstein & Ciszek, 2002). Cell culture studies (Hayflick & Moorhead, 1961; Shay & Wright, 2000) and cell division theory thus predict a negative correlation between telomere length (henceforth TL) and advancing age (Aubert & Lansdorp, 2008; Vaiserman & Krasnienkov, 2020). Studies exploring the diversity of TL and dynamic patterns across species have led to contrasting results; for example, TL has generally been positively associated with maximum lifespan in mammals (Gomes et al., 2011), but not in birds (Tricola et al., 2018). Five divergent relationships between TL and age have so far been observed: (i) a decrease in TL with increasing age (e.g., Allsopp et al., 1992), although some species, including humans (Aubert et al., 2012; Lin et al., 2019), show a more rapid decline in young age cohorts compared to older age cohorts (Froy et al., 2021); (ii) a rapid increase in TL in neonates/juveniles followed by no change in TL with increasing age (e.g., Ujvari & Madsen, 2009); (iii) a curvilinear relationship between TL and age—that is, an increase in TL during the first years of life followed by a decrease in TL in older individuals (e.g., Ujvari et al., 2017); (iv) no change in TL with increasing age (e.g., Plot et al., 2012); and (v) an increase in TL with increasing age (Adwan Shekhdem et al., 2019; for a recent review see Remot et al., 2021).

The diverse pattern in vertebrate telomere dynamics is influenced by a plethora of genetic and environmental factors, including genetic variation and inheritance (Bauch et al., 2019)—but see Dugdale and Richardson (2018)—as well as paternal age (De Meyer et al., 2014; Eisenberg et al., 2019) and parental care effects (Viblanec et al., 2020). Environmental, developmental and psychological stress (Aydinonat et al., 2014; Boonekamp et al., 2014; Epel et al., 2004; Haussmann & Heidinger, 2015; Kotschal et al., 2007), increased production of reactive oxygen species (ROS) (Von Zglinicki, 2002) and/or the organism's reduced capacity for oxidative damage defences have been linked to telomere shortening (Monaghan & Haussmann, 2006; Richter & von Zglinicki, 2007; Von Zglinicki, 2002)—but see Boonekamp et al. (2017) and Richter and von Zglinicki (2007). Limited resource availability, exposure to a harsher climate and/or weather variations between years have been shown to cause enhanced shortening of telomeres (Foley et al., 2020; Wilbourn et al., 2018), while longitudinal studies (Fairlie et al., 2016; van Lieshout et al., 2019) and meta-analyses (Chatelain et al., 2020) have revealed highly dynamic telomere changes during individual lifespan (reviewed in Olsson et al., 2018).

Telomere shortening has been further shown to be accelerated by additional factors such as parasites, pathogenic infections and inflammatory diseases (Blackburn et al., 2015). Infection-induced telomere attrition has been demonstrated by both correlative and

experimental studies in birds (Asghar et al., 2015, 2016; Haussmann et al., 2005) and mammals (Crisuolo, Smith, et al., 2018; Crisuolo, Sorci, et al., 2018; Ilmonen et al., 2008) including humans (Raymond et al., 2014). However, the trend cannot be generalized as Sudyka et al. (2019) found longer telomeres in individuals infected with a parasite in a passerine bird—see also the reviews by Giraudeau, Sepp, et al. (2019) and Monaghan (2014). As resources are not infinite, the trade-off between reproductive effort, immune capacity and somatic maintenance can further alter the extent and direction of telomere attenuation. Enhanced reproductive effort is often associated with higher vulnerability to pathogen infections (Fedorka, 2014; Graham et al., 2011). Hence, the observed links between infection rates and TL have been proposed to be a byproduct of investing into reproductive effort over somatic maintenance (Jacqueline et al., 2016).

Despite the growing interest in infection–telomere interactions, there is very limited knowledge on how transmissible cancers influence telomere dynamics. Transmissible cancers are one of the most intriguing and unexplored host–pathogen systems. The pathogen is a clonal infectious malignant cell line, a rogue cell type that has derived and deviated directly from the host or from a closely related species, and that has crossed the threshold of contagiousness. Although transmissible cancers appear to be rare (but see Dujon, Bramwell, et al., 2020), their evolutionary, ecological and conservation consequences can be major (Dujon, Gatenby, et al., 2020; Russell et al., 2018). An emblematic example of transmissible cancer occurs in the Tasmanian devil (*Sarcophilus harrisii*), whose populations have been dramatically reduced by this infectious disease. Tasmanian devils, the largest extant carnivorous marsupial in the world, once roamed the entire Australian continent, but have been restricted to the island state of Tasmania over the last 3000 years (Brown, 2006; White et al., 2018). Tasmanian devils are sexually dimorphic, males are larger than females, with an average body length of 65 and 57 cm, and an average weight of 8 and 6 kg for the two sexes, respectively (Guiler, 1970).

Prior to the devil facial tumour disease (DFTD) epidemic, devils reached sexual maturity at an age of 2 years and would live for up to 5, or sometimes 6 years in the wild (Jones et al., 2008). Approximately 10 years after the emergence of the transmissible cancer in north-eastern Tasmania in 1996, devils were able to reach sexual maturation at an age of 1 year due to higher resource availability but rarely survived beyond 3 years of age (Jones et al., 2008). In 2014 a second cancer, DFT2, was detected which is currently restricted to the D'Entrecasteaux Channel area, a peninsula in Tasmania's southeast (Pye, Pemberton, et al., 2016).

Although devils have functional immune systems with complete immune gene repertoires, DFTD has until very recently killed most devils with visible tumours (Hamede et al., 2021). However, over the last decade devils have begun to show adaptations to the disease, that is changes in allele frequency of genes involved in immune function and tumour suppression (Epstein et al., 2016; Fraik et al., 2020; Margres et al., 2018; Stahlke et al., 2021), devils surviving longer with visible tumours (Hamede et al., 2021), variation in DFTD infection

with natural antibody prevalence (Ujvari et al., 2016) and immune responses culminating in tumour regression in some devils (Margres et al., 2018; Pye, Hamede, et al., 2016). The observed population reductions together with the local emergence of host adaptations demonstrate that DFTD has an eco-evolutionary impact on the host (Russell et al., 2018), putting this system in the limelight for studies on the dynamics of interactions between transmissible cancers and telomere maintenance. Previous studies to this effect found extreme TL dimorphism in Tasmanian devils, potentially driven by the paternal germline (Bender et al., 2012), and uniformly short telomeres in DFTD tumours (Bender et al., 2012; Ujvari et al., 2012). A recent study found a positive association between TL and age at first infection with DFTD, and proposed that individuals with longer telomeres were less susceptible to transmission and/or progression of the cancer (Smith et al., 2020). Smith et al. (2020) concluded that individuals with longer telomeres may be able to sustain oxidative damage, and control tumour growth more efficiently than devils with shorter telomeres. Cross-sectional analyses of telomere dynamics may, however, be confounded by artefacts arising from cohort effects and selective appearance/disappearance of individuals (Nussey et al., 2014).

The present study is therefore based on longitudinal data and our aim was to investigate (i) individual temporal changes in TL, (ii) how parasite load, season, sex and DFTD may affect devil telomere dynamics, and (iii) how the combination of these extrinsic and intrinsic factors may influence DFTD epidemiology.

2 | MATERIALS AND METHODS

Tasmanian devils were captured at West Pencil Pine (WPP) in northwest Tasmania (41°31' S, 145°46' E) between 2012 and 2019. DFTD first appeared at this site in 2006, and the population has been regularly monitored since with a mark-recapture framework over 10 nights per capture session (see Hamede et al., 2012). The capture sessions were selected to coincide with major life-history events of the devils: in February (summer), when the juveniles are dispersing, and the start of the mating season; in May (autumn), around the end of the mating season; in August (winter), when females usually have young in their pouches; and in November (spring), when females are usually in late lactation (Hamede et al., 2012). All devils were individually marked with a microchip (Allflex), and their sex, age, body size (i.e., head width) and body mass were determined following Hamede et al. (2015). The number of ticks (*Ixodes* spp.) was also assessed, but identifying the ticks to species level was beyond the scope of the current study. A survey conducted at the study site in 2018 indicated the common marsupial tick (*Ixodes tasmani*) being the sole species present (Munns, 2018).

Disease status was assessed by histopathological examination of tumour biopsies or by visual inspection of tumours (Hamede et al., 2015). Since our study is focused on longitudinal telomere dynamics, only animals with a minimum of one recapture were included into the analyses. Altogether 43 (27 females and 16 males)

Tasmanian devils (ranging in age from 11 to 71 months) were analysed. Two of the devils, one female and one male, were infected when first sampled and 23 of the devils were tumour free during the first capture and subsequently developed DFTD (16 females and seven males). As these are wild devils, it was not possible to sample all individuals at all time points. Samples were taken from individuals whenever they were captured.

Approximately 0.5 ml of blood was collected using a needle prick to a peripheral ear vein and stored in 70% ethanol. DNA was isolated from leucocytes by phenol-chloroform extraction, and DNA quality and quantity was determined by using a Qubit 2.0 fluorimeter (ThermoFisher) and Qubit dsDNA High Sensitivity Assay Kit.

2.1 | Quantitative PCR of relative telomere copy number

Due to the unique telomere biology of Tasmanian devils, TL cannot be quantified using telomere restricted fragment (TRF) methods (Ujvari et al., 2012) and we instead used quantitative polymerase chain reaction (qPCR) of relative telomere copy number as proxy of relative TL variation as described by Cawthon (2002). The use of qPCR in determining TL may result in flawed estimates (Eastwood et al., 2018; Lin et al., 2019; Nettle et al., 2021). To reduce the risk of possible inaccurate results, we adhered to the requirements listed by Bustin et al. (2009) when conducting the qPCR analyses, and only samples where all triplicates fell within the range of ± 0.5 Cq were retained in downstream analyses. To reduce possible effects of interplate variation, the id of the plate on which the analyses for TL were run was entered as a random intercept in the analyses.

Telomere-specific primers were adopted from Bize et al. (2009): forward and reverse telomere primers were 5'-CGGTTTGTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3' (Tel1b) and 5'-GGCTTG CCTTACCCTTACCCTTACCCTTACCCTTACCCT-3' (Tel2b), respectively. We chose the primers designed by Bize et al. (2009) over those described by Cawthon (2002) as we were able to achieve a standard curve with $R^2 > .99$ with the Bize et al. (2009) primers. For internal control, we used the single-copy gene RPLP0 as described by Ujvari et al. (2012). The qPCRs were performed on the CFX Connect Real-Time PCR Detection System (Bio-Rad) in 15 μ l total volume, containing 10 μ l Bio-Rad SsoAdvanced universal SYBR Green Supermix (2 \times) (Bio-Rad), 0.2 or 0.5 μ M forward and reverse primers (Telomere and RPLP0 respectively) and 2 μ l of genomic DNA (gDNA) in 0.01 ng μ l⁻¹ concentration in the telomere reaction and 2 μ l of gDNA in 1 ng μ l⁻¹ concentration in the RPLP0 reactions. Two microlitres of a standard sample was run on every plate at a concentration of 0.01 ng μ l⁻¹ for the telomere standards, and 1 ng μ l⁻¹ for the RPLP0 standards. qPCR conditions were established according to the manufacturer's protocol: 95°C for 30s (denaturation), followed by 45 cycles of 95°C for 10 s and 60°C for 30s (annealing temperature: AT). Fluorescence signal was acquired at the annealing temperature. To evaluate the specific amplification, a final melting curve analysis (from AT up to 95°C) was added under continuous fluorescence measurements.

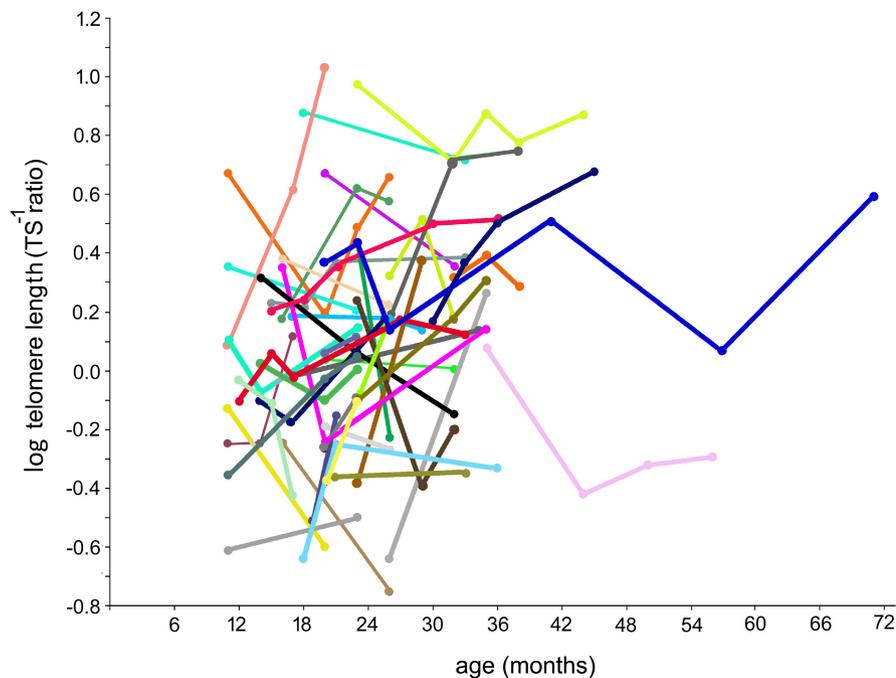


FIGURE 1 Temporal variation in telomere length of 43 Tasmanian devils. Colours depict different individuals

Standard curves were generated using serial 1:5 dilutions of a composite sample containing equal parts of DNA from all blood extracts. All dilutions were run in triplicate. Standard curves had an $R^2 > .994$ (RPLPO reaction: $R^2 = .995$ and Telomere reaction $R^2 = .994$) and PCR efficiencies between 96.7% (RPLPO) and 111.1% (Telomere). The standard curves contained five dilutions from the dilution series with a linear dynamic range of at least three orders of magnitude. All samples were run in triplicate, and all Cq values for unknowns fell within the linear quantifiable range of the appropriate standard curves (Supporting Information). Normalized copy number variation of the target gene compared to the reference gene was calculated by a Pair Wise Fixed Reallocation Randomisation Test as described by Pfaffl et al. (2002). See Supporting Information for standard curves and melting curves.

2.2 | Statistical analyses

Except for the analysis of the temporal variation in individual TL, which was conducted in JMP 5.1 (SAS, 2005), all the remaining analyses were conducted using R Version 4.0.3 integrated into RSTUDIO version 1.3.1093 (R Core Team, 2018). We used linear mixed effect models (package ; Bates et al., 2015) to evaluate the relationship between TL and the explanatory variables age (months), sex (male or female), presence/absence of DFTD, season (summer, autumn, winter and spring), body mass and the number of ticks. Head width was not used in the analysis since it was highly correlated with body mass ($r = .88$). Prior to analysis, TL was \log_{10} -transformed to obtain normality of the data. Since, as pointed out in the Introduction, a multitude of different relationships have been found between TL and age to date, we entered in the analysis the untransformed age, the \log_{10} -transformed age and the square of age. By doing so we

allowed for the detection of linear, accelerating, decelerating and curvilinear relationships between TL and age. Thus, a total of eight explanatory variables were investigated, of which the continuous variables were all centred and scaled prior to their use in the analysis. The id of the individual devils investigated was entered as a random intercept and, as mentioned above, to reduce possible effects of interplate variation the id of the plate on which the analyses for TL were run was also entered as a random intercept in the analyses. Using the function dredge within package MUMIN (Barton, 2020) we evaluated all possible combinations of explanatory fixed-effect variables including their two-way interactions using a maximum of 12 explanatory variables in any model to avoid overfitting. In this analysis we respected marginality constraints, and two-way interaction terms were thus only considered in combination with their two respective main effects. The Akaike information criterion for small samples (AICc) was used to rank models with only models within 7 AIC units of the top model (i.e., $\Delta AICc \leq 7$) being retained for further analyses.

To evaluate the chance of developing DFTD in relation to (\log_{10} -transformed) TL we conducted a Cox proportional hazard analysis using the function coxph in the R-package Survival (Therneau, 2021) using body mass (\log_{10} -transformed) age and sex as additional explanatory variables alongside TL and considering id of individual devils in the analysis.

3 | RESULTS

The intra- and interassay CVs were 1.06% ($n = 14$) and 0.49% ($n = 14$) respectively, suggesting small errors in qPCR estimates of devil TL. Our analyses, however, revealed a substantial temporal variation in individual TL (Bartlett test $p = .048$, $df = 42$; Figure 1). An exhaustive

explorative analysis of TL in relation to the set of eight explanatory variables, including their interactions, revealed that only age, and age in all three ways it was entered in the analysis (i.e., untransformed age, age squared and log of age), was significantly and positively affecting TL (Table 1). According to Burnham and Anderson (2002), the level of empirical support for a model is substantial if its $\Delta AICc$ is within 0–2 units, with higher $\Delta AICc$ of up to 7 having considerably less support. Of the models with $\Delta AICc \leq 7$ the maximum number of terms was two and none contained interaction terms. Models up to a $\Delta AICc$ of 2 included, age, age², log(age), DFTD and sex, with models up to a $\Delta AICc$ of 7 additionally including body mass and ticks. However, none of the non-age parameter estimates of the explanatory variables reached significance, with only females and animals with DFTD showing marginally significant longer telomeres (Table 1). To summarize these significant effects of age and marginally significant effects of sex and DFTD on TL we plotted TL in relation to these three variables in Figure 2. Given these weak relationships, it is unsurprising that the proportional hazard analyses yielded no significant effect of TL on developing DFTD (Table 2). Only age had a significant effect with older animals having an increased risk of developing DFTD (i.e., their hazard ratio was >1).

4 | DISCUSSION

As blood cells offer a minimally invasive sampling option and insight into organismal telomere dynamics and have been shown to be an adequate proxy of tissue-specific TL (Demanelis et al., 2020), we investigated the relative TL from leucocytes, which consist

of a diverse pool of cells. Seasonal variation in the ratio between peripheral blood leucocytes, such as neutrophils, basophils, eosinophils, monocytes and lymphocytes, is known to be present in humans (Maes et al., 1994), and these cells may also display variation in TL (Aubert et al., 2012; Fairlie et al., 2016). Thus, in theory, temporal changes in leucocyte composition could potentially result in apparent changes in TL, which needs to be considered in the interpretation of our results. For example, lymphocytes may have longer telomeres than neutrophils (as seen in baboons; Beaulieu et al., 2017) and this might lead to an apparent decrease of leucocyte TL with advancing age. Importantly, in devils, the neutrophil/lymphocyte ratio increases in both males and females up to an age of 2 years (Cheng et al., 2017), but this change in ratio did not result in any concomitant significant change in relative TL (Cheng et al., 2017). We therefore consider it unlikely that the high individual variation and the age-dependent increase in devil TL were caused by temporal changes in leucocyte composition. The observed high temporal variation in devil individual TL mirrors findings obtained in other longitudinal studies of telomere dynamics such as in Soay sheep (*Ovis aries*; Fairlie et al., 2016) and greater mouse-eared bats (*Myotis myotis*; Foley et al., 2020). In the latter species, telomere dynamics were correlated with seasonal changes in climatic conditions (i.e., spring transition, average temperature, minimum temperature, rainfall) (Foley et al., 2020). In free-ranging mandrills (*Mandrillus sphinx*) the leucocyte profile varied seasonally, with lower lymphocyte prevalence during the long dry season, which was concomitantly associated with shorter telomeres (Beaulieu et al., 2017). In contrast, we did not observe any effect of season on individual devil telomere dynamics, and it is thus unlikely that changes

TABLE 1 Models with $\Delta AICc < 7$, ranked in ascending order by $\Delta AICc$, investigating the relationship between telomere length and a series of explanatory variables

| Explanatory variable 1 | <i>p</i> (estimate ± SE) | Explanatory variable 2 | <i>p</i> (estimate ± se) | <i>Df</i> | AICc | $\Delta AICc$ | Model weight |
|------------------------|--------------------------|------------------------|--------------------------|-----------|---------|---------------|--------------|
| log(age) | .02 (.03 ± .01) | | | 5 | -146.31 | 0 | 0.15 |
| sex | .08 (-.07 ± .04) | | | 5 | -146.27 | 0.04 | 0.15 |
| age | .02 (.03 ± .01) | | | 5 | -146.14 | 0.17 | 0.14 |
| DFTD | .07 (.04 ± .02) | | | 5 | -145.38 | 0.92 | 0.09 |
| age ² | .04 (.02 ± .01) | | | 5 | -144.71 | 1.59 | 0.07 |
| DFTD | .07 (.04 ± .02) | sex | .09 (-.07 ± .04) | 6 | -141.52 | 4.79 | 0.01 |
| log(age) | .03 (.02 ± .01) | sex | .15 (-.06 ± .04) | 6 | -141.49 | 4.82 | 0.01 |
| age | .04 (.02 ± .01) | sex | .15 (-.06 ± .04) | 6 | -141.33 | 4.98 | 0.01 |
| mass | .91 (.00 ± .01) | | | 5 | -140.8 | 5.5 | 0.01 |
| ticks | .72 (.00 ± .01) | | | 5 | -140.68 | 5.62 | 0.01 |
| age ² | .07 (.02 ± .01) | sex | .13 (-.06 ± .04) | 6 | -140.14 | 6.16 | 0.01 |
| log(age) | .00 (.04 ± .01) | mass | .12 (-.02 ± .01) | 6 | -139.87 | 6.43 | 0.01 |
| age | .19 (.05 ± .04) | age ² | .54 (-.02 ± .04) | 6 | -139.48 | 6.83 | 0.00 |

Note: All these top models had a maximum of only two explanatory variables of which the *p* values are depicted (with the parameter estimates and SE values in parentheses). Explanatory variables significantly contributing to TL have emboldened *p* and estimate values. Parameter estimates for sex are for males (with females set to zero). All parameter estimates for continuous explanatory variables in the top models had a positive sign with the exception of age² in combination with age, and body mass in combination with log(age) (i.e., two lowest ranked models at the bottom of the table). For the explanatory factors, females and animals with DFTD tended to have longer TLs though these effects never reached significance.

in leucocyte composition or climatic conditions were underpinning the observed individual variation in devil TL. Alternative mechanisms that may explain the observed periodic increase in telomere copy numbers are intermittent activation of telomerase enzyme and/or alternative pathways that facilitate elongation of telomeres (Cesare & Reddel, 2010; Weng, 2012). However, currently, we lack data to investigate whether the temporal variation in individual devil TL was caused by temporal variation in telomerase activity. As previous studies found telomerase reverse transcriptase (TERT), the catalytic subunit of telomerase, to be expressed at low levels in normal devil tissues, such as testis, spleen and lymph nodes (Bender et al., 2012; Ujvari et al., 2012), it is possible that the production of this enzyme is periodically reactivated throughout the life of Tasmanian devils.

No significant difference in male and female devil TL was observed, although there was a tendency for females having longer telomeres compared to males. Interestingly, Bender et al. (2012) observed differences in TL between homologous chromosomes, with longer telomeres on a haploid set of chromosomes inherited from the male parent, and shorter ones from the female parent. The authors proposed a germline resetting model as the underlying mechanism, that is telomere copy amplification in the male germline and diminution in the female germline as a mechanism to establish

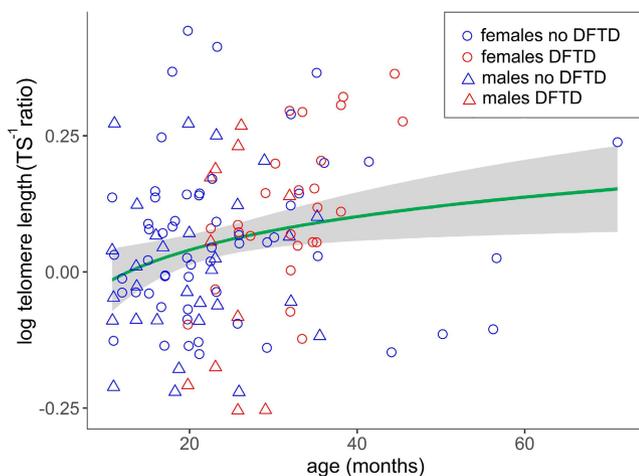


FIGURE 2 Plot of log telomere length (TL) in Tasmanian devils against their age, with symbol colour and shape depicting sex and the presence/absence of DFTD (i.e., displaying all [near-] significant explanatory variables for TL; see Table 1). The relationship between log telomere length and log age is depicted by the green line, including its 95% confidence interval (grey shading), representing the top model from Table 1

| Explanatory variable | Coefficient | SE | Hazard ratio (and 95% confidence interval) | p |
|----------------------|-------------|-------|--|------|
| log(telomere length) | 1.083 | 1.090 | 2.95 (0.27–32.69) | .377 |
| log(age) | 3.701 | 1.081 | 40.47 (3.24–505.08) | .004 |
| body mass | 0.064 | 0.162 | 1.07 (0.81–1.40) | .645 |
| sex (male) | 0.171 | 0.516 | 1.19 (0.46–3.09) | .725 |

TABLE 2 Results of the cox proportional hazard model for the development of DFTD over time in relation to the \log_{10} of telomere length, \log_{10} of age, body mass and sex

dimorphism in the embryo. As our technique was not able to differentially measure TL between homologous pairs of chromosomes, we were not able to establish whether female devils have on average longer telomeres on both haploid sets of chromosomes, or if the pattern is driven by particularly longer telomeres inherited from the male parent. In the context of other vertebrates, our results were not surprising as highly divergent distribution of sex-specific TL has been observed across species; that is, in some species TL is longer in females, while in others males have longer TL than females (Barrett & Richardson, 2011). Given the substantial among-species intersexual variation in TL observed, the possible adaptive significance of this variation is presently unresolved (Barrett & Richardson, 2011; Remot et al., 2020).

Our observation of an age-dependent increase in TL mirrors the results obtained in male long-lived naked mole rats (*Hetercephalus glaber*; Adwan Shekhidem et al., 2019). However, in contrast to naked mole rats, TL increased with age in both male and female devils. The increase of TL in male naked mole rats has been suggested to be caused by an upregulation of telomerase activity (Adwan Shekhidem et al., 2019; but see Foley et al., 2020). Similar telomerase-induced TL compensation mechanisms have been observed in human centenarians (Atzmon et al., 2010) and in human leucocytes (Matsubara et al., 2006). As TERT expression has been detected in devil somatic tissues (Bender et al., 2012; Ujvari et al., 2012), we tentatively suggest that the increase in TL with age in Tasmanian devils may be due to upregulated telomerase activity during the ageing process despite potentially also causing elevated susceptibility to cancer (Hahn & Meyerson, 2001; McNally et al., 2019), as observed in captive devils (Griner, 1979).

Apart from causing physical damage to the host by sucking blood, tick infections have also been shown to cause local inflammations inducing both innate and adaptive immune responses (Wikel, 2013). Importantly, tick infestation frequently results in transmission of pathogens such as viruses (Jones et al., 1989), bacteria (Zeidner et al., 2002) and protozoans (Karasartova et al., 2018; Pereira et al., 2016). As inflammation-inducing parasites and pathogens have been shown to result in increased telomere attrition in birds (Asghar et al., 2015, 2016; Haussmann et al., 2005) and mammals (Crisuolo, Smith, et al., 2018; Crisuolo, Sorci, et al., 2018; Ilmonen et al., 2008), including humans (Raymond et al., 2014), we tested whether tick infestation had any effect on TL. We observed a lack of correlation between tick numbers and TL in devils, which may be explained by several factors: (i) ticks might not invoke an inflammatory response in Tasmanian devils, (ii) tick numbers on devils

can vary vastly with age, season and body conditions, and (iii) the rapid fluctuations in tick numbers may not evoke a response that can be detected in the TL of leucocytes. Further studies investigating the associations between TL, telomerase activity and inflammatory markers as well as pathogens with ticks as vectors will be necessary to determine the hidden cost of infection on telomere dynamics.

In contrast to a cross-sectional study investigating associations between DFTD prevalence and TL conducted by Smith et al. (2020), our longitudinal results showed a tendency for longer telomeres in DFTD-affected individuals compared to healthy devils. However, given that DFTD is also more prevalent in older animals, and TL was also positively correlated with age, the increase of TL with age and longer TL in DFTD-infected animals could be two sides of the same coin. Thus, we need to acknowledge that one of these two relationships could be spurious. However, when further investigating the possible relationship between DFTD and TL, we found that while Smith et al. (2020) noted a tendency for devils with shorter telomeres to become infected with DFTD at a higher rate, we did not observe such an association (no significant relationship between TL and hazard ratios was detected in our data set). The lack of concordance between our and Smith et al.'s (2020) results may arise due to several factors. First, although Smith et al. (2020) noted a negative association between TL and DFTD hazard ratios, they specify that these results were not significant, probably due to the small sample sizes used in their data set. Second, in our study we used whole blood samples collected between 2012 and 2017, while Smith et al. (2020) used ear biopsies collected between 2006 and 2014 (at the same location). Although measuring relative TL from blood samples has been accepted as adequate proxy of tissue-specific TL (Demanelis et al., 2020; Rollings et al., 2019), the concentration and composition of different cell types may have been different between ear biopsies and whole blood samples, potentially causing deviations between the results of the two studies. Since our and Smith et al.'s (2020) studies cover different periods of this epidemic, it is possible that we are observing different host responses to infection pressure (that might have originated from different infection and mortality rates at different time points). Finally, in our study we focused only on one population of Tasmanian devils, WPP, from where we had the opportunity to obtain longitudinal data. In contrast, Smith et al. (2020) analysed cross-sectional data across three locations, WPP, Freycinet and Narawntapu, and noted devils having longer telomeres at WPP, indicating location-specific telomere dynamics. Thus, to decipher the associations between TL and disease risk, relative TL should be measured by using longitudinal data across the entire period of an epidemic and across different populations that have been exposed to the disease for different lengths of time.

5 | CONCLUSION

In accordance with other studies, we show the enormous complexity of TL maintenance across the lifetime of organisms and within a vertebrate species. Our findings support previous observations, namely that the associations between TL and intrinsic and

environmental factors probably differ between most species based on their life history and reproductive strategies. Furthermore, we demonstrate the importance of longitudinal sampling, potentially across different tissue types, when investigating the factors driving telomere dynamics in the face of disease epidemics. We highlight the importance of extending any further studies to incorporate the measurements of telomerase activity when attempting to understand lifetime telomere dynamics.

AUTHOR CONTRIBUTIONS

Beata Ujvari: conceptualization, data generation and curation, formal analysis, methodology, writing—original draft, secured funding. **Marcel Klaassen:** formal analysis, writing—original draft. **Nynke Raven:** data generation and curation, writing—original draft. **Antoine M. Dujon:** writing—original draft. **Geordie Jennings:** data generation and curation, writing—original draft. **Frédéric Thomas:** writing—original draft, secured funding. **Rodrigo Hamede:** fieldwork, data collection and curation, investigation, writing—original draft, secured funding. **Thomas Madsen:** conceptualization, data analyses, writing—original draft.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and scripts have been archived under: <https://doi.org/10.5061/dryad.3tx95x6k9>.

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SUPPORTING INFORMATION

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

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