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Telomere length is a susceptibility marker for Tasmanian devil facial tumor disease

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Abstract

Telomeres protect chromosomes from degradation during cellular replication. In humans, it is well-documented that excessive telomere degradation is one mechanism by which cells can become cancerous. Increasing evidence from wildlife studies suggests that telomere length is positively correlated with survival and health and negatively correlated with disease infection intensity. The recently emerged devil facial tumor disease (DFTD) has led to dramatic and rapid population declines of the Tasmanian devil throughout its geographic range. Here, we tested the hypothesis that susceptibility to DFTD is negatively correlated with telomere length in devils across three populations with different infection histories. Our findings suggest telomere length is correlated with DFTD resistance in three ways. First, devils from a population with the slowest recorded increase in DFTD prevalence (West Pencil Pine) have significantly longer telomeres than those from two populations with rapid and exponential increases in prevalence (Freycinet and Narawantapu). Second, using extensive mark-recapture data obtained from a long-term demographic study, we found that individuals with relatively long telomeres tend to be infected at a significantly later age than those with shorter telomeres. Third, a hazard model showed devils with longer telomeres tended to become infected at a lower rate than those with shorter telomeres. This research provides a rare study of telomere length variation and its association with disease in a wildlife population. Our results suggest that telomere length may be a reliable marker of susceptibility to DFTD and assist with future management of this endangered species.

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Keywords

telomeres; infectious disease; devil facial tumor disease; DFTD; wildlife health

Introduction

Recent studies have suggested that environmental stressors, such as emerging infectious diseases and increased population fragmentation, can result in accelerated telomere degradation in wild populations (Mizutani et al., 2013; Romano et al., 2013). Telomeres are DNA-protein complexes that cap the ends of all eukaryotic chromosomes, thereby protecting chromosomes from degradation during cell replication (Blackburn, 1991). With each round of cell division, several telomeric repeats are lost (Chan and Blackburn, 2004). Under normal conditions, when telomeres become critically short, apoptosis (terminal cell cycle arrest) is induced via the p53 regulatory pathway. Cancerous cells can form when this pathway is avoided, and genome instability is facilitated through rounds of breakage-fusion-bridge (BFB) cycles, that result in uneven chromosome separation and breakage during mitosis (Ducray et al., 1999). Numerous empirical studies in humans have identified associations between telomere length and risk of age-related diseases such as cancer. Two meta-analyses have determined a significant association between short telomeres and increased risk for developing different cancer types, including bladder, renal, and gastric cancers (Ma et al., 2011; Wentzensen et al., 2011).

Although primarily used as a marker for cancer risk and overall health in human studies, there is mounting evidence that telomere length is positively correlated with performance and survival in wildlife species. For instance, one study showed that tree swallows with short erythrocyte telomeres in early life have lower survival than those with longer telomeres (Haussmann et al., 2005). Similarly, zebra finches with longer telomeres measured at 25 days old lived longer than those with short telomeres (Heidinger et al., 2012).

Stress caused by fluctuation of environmental conditions has been shown to accelerate telomere degradation in wild vertebrates. American redstarts (*Setophaga ruticilla*) that wintered in low-quality habitats had significantly higher rates of telomere shortening relative to redstarts in high-quality winter habitats (Angelier et al., 2013). Large climatic fluctuations, such as El Niño, are expected to increase in frequency due to climate change. The El Niño in 2010 was associated with favorable foraging conditions in Japanese black-tailed gulls (*Larus crassirostris*) and with an increase in average telomere length (Mizutani et al., 2013).

Infectious diseases are also associated with telomere dynamics in natural populations. For example, Great reed warblers (*Acrocephalus arundinaceus*) infected with chronic avian malaria had accelerated telomere degradation relative to uninfected birds (Asghar et al., 2015). In another study, badger immune cell telomere lengths were correlated with disease status; animals in early stages of infection by bovine tuberculosis (bTB) had longer immune cell telomeres than uninfected individuals, but badgers in advanced stages of bTB infection had shorter telomeres than badgers in early stages of infection (Beirne et al., 2014). These

studies highlight the potential for telomeres to serve as an indicator of health status of wild populations of vertebrates.

One proposed explanation for these and similar associations is greater replicative potential in cells having long telomeres relative to those with short telomeres. Although telomerase, the enzyme that increases telomere length, is typically downregulated as a means of tumor suppression, under some conditions telomerase can be upregulated and telomere repair can occur (Webb et al. 2013). This increased cell survivability is proposed to lead to enhanced tissue and organ function (Haussman et al., 2005). Particularly with regard to infectious diseases, possessing a high replicative potential would be especially important for maintenance and proliferation of immune system cells. Indeed, shortened leukocyte telomeres is associated with compromised immune function (Weng 2012).

Here, we focus on the endemic Tasmanian devil (*Sarcophilus harrisi*), an iconic marsupial threatened with extinction due to a transmissible cancer known as devil facial tumor disease (DFTD). The cancer was first detected in northeast Tasmania in 1996 and has since spread across the majority of the island of Tasmania, thereby impacting nearly the entire species' geographic range (Hamede et al., 2015; McCallum et al., 2009). The cancer is spread among individual devils through direct contact, usually biting, during social interactions such as competition for food or territory (Hamede et al., 2013). Tumor cells are directly transferred to a new host and evade detection by the Tasmanian devil immune system by downregulating expression of the devil major histocompatibility complex (MHC) (Siddle et al., 2013). DFTD is nearly 100% fatal, causing declines exceeding 90% in most affected populations, with a species-wide decline greater than 80% (Hamede et al., 2012; McCallum et al., 2009). Concomitant with these declines is a collapse in population age structure, with older individuals (3–5 years old) typically disappearing from populations with high DFTD prevalence (Hamede et al., 2015; Jones et al., 2008). Even in the DFT-free environment, Tasmanian devils are fairly short lived, with a life expectancy of only 4–5 years. One reason for this short lifespan could be that telomeres shorten quickly in this species, leading to widespread apoptosis and senescence. Indeed in mammals, telomere length is positively correlated with maximum lifespan (Gomes, 2011). Additionally, telomere length may show promise as a marker for cancer prognosis in devils based on results from multiple human studies that show that cancer survival rate is positively correlated with telomere length (Weischer et al., 2013; Zhang et al., 2015).

Here, we studied telomere length variation in uninfected and infected Tasmanian devils from three geographically disparate sites. Our study had three aims. First, we tested whether there were differences in telomere length between uninfected male and female devils. Previous work has shown that the family Dasyuridae (carnivorous marsupials) show extreme variation in telomere length between chromosome homologs and hypothesized this was the result of variation in telomere length in gametes, with sperm having longer telomeres than eggs (Bender et al., 2012).

Second, we tested for geographic variation in telomere length among populations; because devils are highly inbred (Miller et al., 2011), telomere length variation may be very low. On the other hand, Tasmanian devils have shown variation in population-level responses to

DFTD. One population in particular, West Pencil Pine (WPP; Fig. 1), had a unique epidemiological progression of DFTD following emergence (Hamede et al., 2012, 2015). Instead of an exponential increase in prevalence typically observed in other populations, DFTD infection progressed slowly and population stability was observed for several years after disease introduction (Hamede et al., 2013, 2015). Additionally, the first evidence of devil antibody production and even complete tumor regression was observed in this population, suggesting a relationship between host health status and disease progression (Pye et al., 2016).

Third, we tested whether telomere length was correlated with DFTD susceptibility. We utilized phenotypic information from long-term mark-recapture studies to: a) compare telomere lengths of diseased individuals to those that never developed DFTD; and, b) determine whether telomere length was correlated with age at which individuals first became infected, as well as tumor growth rate once infected. We hypothesized that telomere length would be positively correlated with age of first infection and negatively correlated with tumor growth rates.

Materials and Methods

Field collection

Tasmanian devils were trapped using custom-built traps constructed of 300 mm polypropylene pipe. All traps were baited with meat. Trapping sessions were carried out with 40 traps over 7–10 consecutive nights in a capture–mark–recapture framework. Traps were checked daily beginning at dawn; details of field methods were previously described (Hamede et al., 2015). Following initial capture, devils were individually tagged with microchip transponders (Allflex NZ Ltd, Palmerstone North, New Zealand) and 2mm ear biopsies (Hawkins et al., 2006) were taken to provide tissue for genetic analyses. Devils were aged using a combination of head width, molar eruption, molar tooth wear, and canine over-eruption. Most individuals were trapped as juveniles (<1 year) and, therefore, the age was known. DFTD status was categorized from histopathological confirmation of tumor biopsies (Pye et al. 2016) and/or confirmatory PCR analysis (Kwon et al. 2018).

Sampling design

To address the first two aims of the study (whether there were differences in telomere lengths between males and females and across geographic variation), we analyzed tissue samples from 132 uninfected Tasmanian devils collected between 1999 and 2013 from three geographically disparate sites across Tasmania (WPP, Freycinet, and Narawntapu; Fig. 1). None of these animals developed DFTD tumors during their lifetime. We included both males and females within each age category (1–5 year olds). Second, to test for relationships between telomere length and disease susceptibility, we analyzed telomere lengths among 132 individuals that showed symptoms of DFTD. Tissue samples were collected from WPP and Freycinet between 2000 and 2014 (Narawntapu was not included due to low sample size). We chose these sites because they contain the most extensive disease recapture data among those studied in Tasmania. Individuals were selected to maximize variation in the age

at which devils first showed signs of DFTD (i.e., age first diseased) to enable tests of whether age first diseased and telomere length were correlated.

DNA extraction

DNA was extracted from ear tissue using the Agencourt DNAdvance Genomic DNA isolation kit (Beckman Coulter, Mount Waverly, Australia). All samples were quantified using a Qubit 2.0 fluorometer (ThermoFisher, Waltham, MA) and Qubit dsDNA High Sensitivity Assay Kit prior to carrying out telomere length measurements using quantitative PCR.

Relative telomere length measurement

Telomere length measurements were conducted in the Cytometry and Telomere Center, Department of Pathology, University of Washington, Seattle - a core facility for quantification of telomeres. Real-time quantitative polymerase chain reaction (qPCR) was used to determine relative telomere lengths. For each sample, two separate PCRs were run: one for telomere amplification and the other for a single copy housekeeping gene, RPLP0 amplification. RPLP0 (acidic ribosomal phosphoprotein P0) was chosen because it is confirmed to be present in only a single copy in the Tasmanian devil genome (Ujvari et al., 2012). This ratio of the PCR-measured quantity of telomere to single-copy housekeeping gene (telomere to single-copy gene ratio; T/S ratio) provided relative measurements of telomeric DNA. The following primers were used: Tel1: 5'-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3'; Tel2, 5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3'; RPLP0 primers: RPLP0_F:5'-CTTCCCGTTCACCAAAGAAG-3'; RPLP0_R:5'-TGTTCTGGACTGGCAAAGTG-3' (from Ujvari et al., 2012). PCR reactions were set up using a Qiagility pipetting robot and carried out on a Rotor-Gene Q (Qiagen Inc.; Hilden, Germany). Each sample reaction had 10 µL total volume with 1.5 ng DNA (diluted to a concentration of 2 ng/ µL for a total volume of 3 µL) and 7 µL of 2X Sybrgreen PCR Mastermix (Qiagen). A HEX-labeled oligo was also included in all reactions as a passive reference dye. All PCR reactions were run under optimal cycling conditions, which was the initial denaturation step at 95°C for 15 min. followed by 40 cycles of amplification at 95°C for 15 sec, and at 56°C for 30 sec for the telomere PCR; cycling conditions for RPLP0 were: denaturation at 95°C for 5 min. followed by 40 cycles of amplification at 95°C for 15 sec and at 58°C for 30 sec. Each PCR run included a standard curve with four points made from half serial dilutions (3 ng to .375 ng) to allow for conversion of Ct (cycle threshold) into nanograms of DNA. The Rotor Gene software (Qiagen, Inc.; Hilden, Germany) was used to normalize Sybrgreen intensity to the HEX passive reference dye. We used third-party software (Rosana2, developed in the Risques lab at University of Washington) to align amplification plots to a baseline height and then calculate Ct based on a fluorescence threshold. All samples were run in triplicate and the median was used for subsequent calculations.

In every PCR, two to three samples were run as controls for normalization between PCR runs. Controls and reproducibility samples were used to determine the correction factor for normalization. Once all data were normalized, the amount of telomeric DNA amplified was

divided by amount of control-gene DNA to produce relative measurements of telomere length (T/S ratio). R^2 for the telomere and control gene standard curves were >0.95 . Efficiency for the telomere and control gene PCRs were >0.93 . Coefficient of variation (CV), which provides a measure of variability between assays, was between 5% and 7.5% which is typical as determined by other groups (Martin-Ruiz et al., 2015). Mean CV for first data set was 0.058; mean CV for the second data set was 0.075; CV between data sets was 0.070, which falls within established values (Glei et al., 2016). Samples above a CV of 0.15 were re-run or discarded.

Statistical analyses

All statistical analyses were performed using the software R 3.2.3 $\times 64$ (R Development Core Team, 2015). To determine whether sex is a useful predictor of telomere length in devils, we conducted a Welch's two sample t-test. To test whether telomere length differed among populations of uninfected devils, we first conducted an ANOVA. This was followed by the use of pairwise t-tests using non-pooled standard deviations and a Bonferroni correction for multiple testing to identify among which populations telomere lengths differed. To test whether telomere lengths differed among populations of infected devils (Freyrcinet and West Pencil Pine), a Welch's two sample t-test was used.

Among the 132 uninfected devils, telomere length was the dependent variable with the following independent variables included as potential explanatory factors: age of individual at time of sample (age), site, year of birth, and all relevant interactions. We constructed a total of 11 linear models using these three hypothesized predictors of telomere length using the `lm` function, and ranked them using `model.sel` in the R package MuMIn (Barton, 2016). Rankings are based on a modified version of Akaike's information criterion (AICc) to account for finite sample size (Symonds and Moussalli, 2010). Any model with a $\Delta AICc$ score ≥ 2 was considered a significantly worse fit than the best fit model. Q-Q plots were constructed to check for normal distribution of residuals. One outlier was removed to meet assumptions of normality for residuals.

To analyze whether telomere length was related to DFTD susceptibility, we constructed three different sets of models. First, 11 linear models were constructed using age at first DFTD infection as the response variable; site, YOB and telomere length (T/S) were used as predictor variables. These 11 models were subsequently ranked based on AICc score. Second, the Cox proportional hazard model was also applied as a test for a relationship between relative telomere length and age of first infection. For this analysis, we included both infected animals and those who were uninfected at the end of the study (at last capture time). Individuals who were uninfected were censored, accounting for the possibility of infection at a later age (after last capture). Once data were censored, the model was conducted using the survival package (`coxph` function; Therneau, 2015) in R. We tested for effects of telomere length, body condition, and mass on $h(t)$ (hazard rate, expected instantaneous rate of infection over time (years)). We determined Kaplan-Meier estimates of infection rate via the `survfit` function in the survival R package. We then compared K-M curves for two groups: individuals with relatively long telomeres (top 10%) and individuals with relatively short telomeres (bottom 10%).

Third, we compared individuals who became diseased versus those who did not. We conducted a logistic regression (glm function; family=binomial; R Development Core Team, 2015) to determine the probability of becoming infected based on the following predictor variables: site, year of birth (to account for difference in disease prevalence each year), telomere length, and age at telomere length collection. A total of 21 models were constructed and ranked by AICc values; all factors were treated as fixed because we were interested in controlling for different levels of each factor.

A final set of 21 models were constructed with tumor growth rate set as the response variable. We included all 45 individuals with tumor growth information, which were comprised of 6 individuals from Freycinet and 39 from WPP. Telomere length, site, age first diseased, year of birth, and biologically reasonable interactions were included as fixed factors. Tumor growth rate was calculated using the following equation, which accounts for its exponential growth:

$$\frac{[\log(\text{total tumor volume at the last capture}) - \log(\text{total tumor volume at the first capture})]}{\text{number of days between first and final volumes}}$$

Results

Uninfected Tasmanian devils

There was no evidence for a difference in telomere length between sexes ($p = 0.3547$; Fig. 2) among uninfected devils. However, individual telomere length was significantly associated with population (labeled *site* in models) ($p = 0.0006$; Table 1). Individuals from WPP had significantly longer telomeres than those from either Narawntapu ($p = 0.0081$) or Freycinet ($p = 0.0011$; Fig 3). Telomere lengths did not differ significantly between Narawntapu and Freycinet ($p = 0.149$). Age at time of sampling and year of birth (an approximation of disease prevalence at each site) each explained additional variance in telomere length after accounting for variance among sites (Table 1).

Telomere length as a predictor for DFTD susceptibility

One model predicting the age at first detection of DFTD in devils was well-supported (Akaike weight = 0.859; Table 2). In this model, age at first detection was predicted by telomere length and an interaction between site and year of birth. A significant, positive association was found between telomere length and age of first infection (Adjusted $R^2 = 0.14$, $p = 1.143e^{-05}$, Fig. 4). The interaction between site and year of birth likely comes from the fact that there was variation in the progression of DFTD in WPP and Freycinet. That is, the former showed a slower increase in disease prevalence from the latter. The hazard ratio for telomere length (i.e., the effect of telomere length on infection rate), although large (0.5298 ± 0.3570), was not significant ($p = 0.0751$). Results from the Cox analysis showed that year of birth was a significant covariate in the models, likely due to correlation between birth year and disease prevalence. Kaplan-Meier curves, typically used to estimate the survival function from lifetime data, were used depict rates of infection for relatively long versus short telomere lengths (Fig. 5). These curves showed that individuals with longer telomeres tended to have lower infection rates than those with shorter telomeres.

One model predicting the probability of contracting DFTD held the majority of support (Akaike weights of top two models: 0.613 and 0.178 respectively; Table 3). In the best-fit model, telomere length was not a predictor of disease status, whereas site and an interaction between YOB and age was. In contrast, no one model predicting tumor growth rate held the majority of support among the candidate set of 21 models (Table 4). The best predictor of growth rate was year of birth (Akaike weight: 0.266), gl.

Discussion

Our study is one of the first to show a relationship between telomere length and disease susceptibility in a wildlife species. Here, we show that relative telomere length in Tasmanian devils is associated with risk of infection by DFTD in three ways. First, devils from WPP, which had the slowest observed increase in DFTD prevalence, have significantly longer telomeres than in Freycinet, which had an exponential increase in prevalence (Hamede et al., 2012). In addition, individuals from WPP were less likely to become infected during their lifetime than those from Freycinet, which had shorter telomeres. Second, we found that telomere length explained about 53% of the variation in propensity to get infected using a hazard model (but these results were marginally non-significant, probably because of small sample size). Third, by analyzing extensive mark-recapture data, we show that individuals with longer telomeres were infected at a significantly later age than those with shorter telomeres. Further, the probability of becoming infected overall is slightly lower for those with relatively longer telomeres. Taken together, these results suggest that telomere length is a potentially important factor in assessing DFTD risk for Tasmanian devils.

Telomere length variation among populations and sexes

We found that individuals at WPP have longer telomeres than Freycinet or Narwantapu. Whereas prevalence of DFTD rises quickly within the first few years of disease introduction, DFTD prevalence at WPP, remained below 5% and population size remained stable during the first five years following DFTD emergence (2006–2011: Hamede et al., 2012). In total, WPP is only one of seven populations studied over the long-term (including Freycinet and Narwantapu) that did not show disease prevalence of at least 50% in 2–3 year olds within five years of its introduction. DFTD strain is likely an important factor in the observed epidemiological patterns at WPP. That is, a tetraploid DFTD strain that first entered the population was found to have a slower growth rate and lower overall mortality rate than a diploid strain that replaced it in 2012 (Hamede et al. 2015).

We also showed that there are no differences in telomere length between males and females, regardless of age, despite previous work that shows Tasmanian devils have dimorphism in telomere lengths between homologous chromosomes (Bender et al., 2012). Telomere length dimorphism is present upon zygote formation and maintained in somatic cells as the organism ages (Bender et al., 2012). The most likely mechanism leading to this is that during gametogenesis telomeres are actively lengthened in the male germline and shortened in the female germline, a general characteristic of the Family *Dasuyridae*. Future studies may benefit from focusing on the relationship of telomere length on different chromosomes

to determine if average telomere length is a result of decrease in chromosome homologs in long versus short telomeres.

Telomere length and disease susceptibility

We found that telomere length was positively associated with age at first infection, after controlling for year of birth, suggesting that individuals with relatively long telomeres became infected with DFTD later in life than those with shorter telomeres. Results from hazard models show a negative association between telomere length and hazard ratio, which is the expected rate of infection. However, these results are not significant, probably because of small sample sizes. Nonetheless, taken together, these results support the prediction that individuals with relatively long telomeres are less susceptible to DFTD than those with shorter telomeres.

We also identified two ways in which telomere length appears to be associated with decreased susceptibility to DFTD- longer telomeres are correlated with a later age of first infection and decreased susceptibility to DFTD. These trends are consistent with results from human disease studies. Two likely explanations for this association are increased immune system function and protection against oxidative damage. It has been well established that immune system function decreases with age, in part because of a reduced number of cell divisions in immune cells with short telomeres, rendering them less efficient in protecting against disease or damage (Haussman et al., 2005). Cells with short telomeres have a reduced replicative potential because they are closer to reaching the Hayflick limit (or the theoretical upper bound of number of mitotic divisions before telomeres no longer protect chromosomes) as compared to cells with long telomeres. In addition, cell replicative potential may affect other organs directly involved in DFTD prognosis, such as skin or internal sites of metastases, as cell division is directly linked to cell loss and renewal. Organs consisting of a large number of senescent cells leads to reduced organ performance. In DFTD, for instance, skin and wound repair at site of the primary infection may be more prone to damage, promoting cancer progression. In addition, certain factors secreted by senescent cells have been shown to promote chronic inflammation, affect tissue homeostasis, and even promote malignant transformation in human and mouse cancers (Coppé et al., 2006; Krtolica et al., 2001). Taken together, individuals with relatively long telomeres may have an advantage of increased tissue and immune system performance. Notably, WPP showed the first signs of antibody production and spontaneous tumor regression in a few individuals (Pye et al. 2016).

A second potential explanation for the association between telomere length and disease susceptibility is a resistance to oxidative damage. Previous studies have shown that oxidative damage is the primary contributor to telomere loss (Monaghan and Haussman, 2006). Telomeres are especially prone to DNA damage by oxidative stress because of their G-rich nucleotide content (Gomes et al., 2011). The relationship between oxidative stress and telomere shortening has been well-established in several species including humans, sheep and mice (Cattan et al., 2008; Richter and von Zglinicki, 2007). Further, long telomeres have previously been shown to be resistant against oxidative damage (Joeng et al., 2004). In vertebrates, it has been found that chronic stress induces oxidative damage (Constantini et

al., 2011), in addition to suppressing immune system function (Glaser and Kiecolt-glaser, 2005). DFTD is likely a significant source of stress in infected devils and could lead to increased oxidative damage, consequently accelerating devil telomere shortening. As such, individuals with relatively long telomeres may be more resistant to oxidative damage and senescence than those with shorter telomeres. Research in humans has shown that senescent cells promote a tumor microenvironment and thereby tumor spread (Campisi et al., 2001; Krtolica et al., 2001). Perhaps it follows, then, that longer telomeres in Tasmanian devils are associated with slower tumor growth than devils with shorter telomeres.

Although we found no evidence that telomere length is associated with tumor growth rate, our sample size was small. Future studies with increased sample sizes may yield greater power to detect a difference. Further, tumor ploidy also likely has important effects on growth rate based on previous findings attributing differences in cell proliferation rates to ploidy (Ganem and Pellman, 2007). In devils, diploid tumors tend to divide and proliferate more rapidly than tetraploid tumors (Pearse et al. 2012), and the observed epidemiological pattern at WPP is consistent with this finding (Hamede et al., 2015). We did not have tumor strain data available for individuals included in this study and were thus unable to determine whether ploidy affected growth rates. Ujvari et al. (2012) propose that the DFTD may be evolving to gain a growth advantage and/or to prevent further genomic instability, a phenomenon found in human cancers (Hackett and Greider, 2002). Thus, it is possible that DFTD strain variation may have a greater effect on tumor growth rates than variation in devil telomere lengths. It is also important to note that telomere length likely varies among tissues within an individual, and future studies may measure telomere length variation in a diversity of tissues.

Conclusion

This study is one of the first to suggest that telomere length can be a useful marker for assessing disease risk among populations. We posit that long telomeres provide an advantage against negative effects of DFTD, including tissue damage and oxidative stress. Future studies should evaluate longitudinal, repeated telomere measures to gain a better understanding of within-individual telomere changes through time, and to provide possible insight into causes and effects of telomere length changes. Nonetheless, the significant associations found here highlight the potential for telomere length to be used as a biomarker for organismal fitness in the face of infectious disease or cancer, concordant with results from previous research on humans and wildlife populations (Haussman et al., 2005; Heidinger et al., 2012).

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Figure 1.
Map of sampling sites in the Australian state of Tasmania.

Figure 2.

The relationship between sex and relative telomere length (y-axis, T/S ratio in disease-free Tasmanian devils across three populations in Tasmania (Narawantapu, Freyinet and West Pencil Pine). Telomere length did not differ among the sexes ($p = 0.3547$). Bars indicate the 95% confidence interval around median value. Individual estimates of tumor length per population are overlain on boxplots and jittered to facilitate visualization.

Figure 3. Relative telomere length found among populations of Tasmanian devils. Telomere lengths differ significantly among populations of uninfected (left: $p = 0.0006$) and DFTD- infected devils (right: $p = 6.54 \times 10^{-6}$). Among uninfected devils, West Pencil Pine individuals have significantly longer telomeres than Narawntapu ($p = 0.0081$) or Freycinet ($p = 0.0011$), but Narawntapu and Freycinet do not significantly differ ($p = 0.149$). Letters indicate among which populations telomere differences are significant. Individual estimates of tumor length per population are overlain on boxplots and jittered to facilitate visualization.

Figure 4. Partial correlation between telomere length and age of first infection.

X-axis is telomere length, whereas the y-axis is the residuals obtained from a regression of age at time of first infection against year of birth (Adjusted $R^2 = 0.1417$, $p = 1.143e^{-05}$).

Figure 5. Kaplan-Meier survival curves to depict infection rates for individuals with relatively short versus relatively long telomere lengths.

The estimated infection rates grouped by relative telomere length ('long'; dashed red line - within the top 10% of telomere length distribution; 'short'; solid blue line- within the bottom 10%). The K-M median estimates of time until infection were 3 years for short telomere group and 4 years for long telomere group.

Table 1.

Linear models predicting relative telomere length in uninfected devils. All fitted models are shown; rankings are based on AICc score. (T/S = relative telomere length; Age = age first diseased, YOB = year of birth).

Model	DF	logLik	AIC_c	AIC_c	Akaike Weight
T/S ~ Site	3	25.893	-43.466	0.000	0.412
T/S ~ Site + Age	4	25.916	-41.347	2.119	0.143
T/S ~ Site + YOB	4	25.909	-41.333	2.133	0.142
T/S ~ Site + YOB * Age	6	27.688	-40.457	3.009	0.092
T/S ~ Site * Age	6	27.572	-40.226	3.240	0.082
T/S ~ Site + YOB + Age	5	26.088	-39.492	3.974	0.057
T/S ~ Site * YOB	6	27.100	-39.282	4.184	0.051
T/S ~ Site * YOB + Age	7	27.249	-37.308	6.158	0.019
T/S ~ Age	2	19.764	-33.337	10.129	0.003
T/S ~ YOB	2	18.531	-30.872	12.594	0.001
T/S ~ Site * YOB * Age	12	28.374	-27.609	15.857	0.000

Table 2.

Linear models predicting age at which devils first become diseased. All fitted models are shown; rankings are based on AICc score. (T/S = relative telomere length; Age = age first diseased, YOB = year of birth).

Model	DF	logLik	AIC_c	AIC_c	Akaike Weight
Age First Diseased ~ Site * YOB + T/S	5	-165.271	343.273	0.000	0.859
Age First Diseased ~ Site * YOB * T/S	8	-164.598	348.803	5.531	0.054
Age First Diseased ~ Site + YOB * T/S	5	-168.225	349.180	5.908	0.045
Age First Diseased ~ Site + YOB + T/S	4	-169.925	350.366	7.094	0.025
Age First Diseased ~ Site * YOB	4	-170.354	351.224	7.952	0.016
Age First Diseased ~ Site + YOB	3	-174.568	357.477	14.204	0.001
Age First Diseased ~ Site + T/S	3	-180.557	369.455	26.182	0.000
Age First Diseased ~ T/S	2	-181.805	369.813	26.540	0.000
Age First Diseased ~ Site * T/S	4	-180.429	371.376	28.103	0.000
Age First Diseased ~ Site	2	-187.097	380.397	37.124	0.000
Age First Diseased ~ YOB	2	-187.099	380.402	37.129	0.000

Table 3.

Logistic regression models predicting probability of infection. All fitted models are shown; rankings are based on AICc score. (T/S = relative telomere length; Age = age first diseased, YOB = year of birth).

Model	DF	logLik	AIC _c	AIC _c	Akaike Weight
Prob Infection ~ Site + YOB * Age	5	-54.008	118.504	0.000	0.613
Prob Infection ~ Site + YOB + Age + T/S	5	-55.243	120.973	2.469	0.178
Prob Infection ~ Site * YOB * Age	8	-52.687	122.573	4.069	0.080
Prob Infection ~ Site + YOB + Age	4	-57.625	123.572	5.068	0.049
Prob Infection ~ Site * YOB * Age * T/S	15	-44.849	123.947	5.442	0.040
Prob Infection ~ Site * YOB + Age	5	-57.320	125.129	6.624	0.022
Prob Infection ~ Site + Age + T/S	4	-59.652	127.627	9.123	0.006
Prob Infection ~ Age	2	-62.036	128.167	9.663	0.005
Prob Infection ~ Site + Age * T/S	5	-59.512	129.511	11.007	0.002
Prob Infection ~ Site + Age	3	-62.012	130.216	11.711	0.002
Prob Infection ~ Site * Age	4	-61.966	132.255	13.751	0.001
Prob Infection ~ YOB	2	-64.947	133.989	15.485	0.000
Prob Infection ~ Site * Age * T/S	8	-58.808	134.815	16.311	0.000
Prob Infection ~ Site	2	-65.958	136.011	17.506	0.000
Prob Infection ~ Site + YOB	3	-65.654	137.501	18.996	0.000
Prob Infection ~ Site + YOB + T/S	4	-64.797	137.917	19.412	0.000
Prob Infection ~ Site * YOB	4	-64.915	138.152	19.648	0.000
Prob Infection ~ Site * T/S	4	-65.145	138.614	20.109	0.000
Prob Infection ~ Site + YOB * T/S	5	-64.757	140.001	21.496	0.000
Prob Infection ~ Site * YOB + T/S	5	-64.771	140.030	21.525	0.000
Prob Infection ~ Site * YOB * T/S	8	-64.185	145.570	27.066	0.000

Table 4.

Linear models predicting exponential tumor growth rates. All fitted models are shown; rankings are based on AICc score. (T/S = relative telomere length; Age = age first diseased, YOB = year of birth). No one model receive the majority of support.

Model	DF	logLik	AIC _c	AIC _c	Akaike Weight
Growth Rate ~ YOB	2	-77.965	162.516	0.000	0.266
Growth Rate ~ Site	2	-78.475	163.535	1.019	0.160
Growth Rate ~ Age	2	-78.535	163.655	1.139	0.150
Growth Rate ~ Site + YOB	3	-77.929	164.857	2.341	0.082
Growth Rate ~ Site + Age	3	-78.266	165.532	3.015	0.059
Growth Rate ~ Site * Age	3	-78.266	165.532	3.015	0.059
Growth Rate ~ Site + YOB + T/S	4	-77.371	166.280	3.764	0.040
Growth Rate ~ Site * YOB	4	-77.588	166.715	4.199	0.033
Growth Rate ~ Site + Age + T/S	4	-77.835	167.208	4.692	0.025
Growth Rate ~ Site + YOB + Age	4	-77.868	167.275	4.759	0.025
Growth Rate ~ Site * T/S	4	-77.919	167.377	4.861	0.023
Growth Rate ~ Site * YOB + T/S	5	-77.083	168.377	5.861	0.014
Growth Rate ~ Site + YOB * T/S	5	-77.164	168.539	6.023	0.013
Growth Rate ~ Site + Age * T/S	5	-77.166	168.542	6.026	0.013
Growth Rate ~ Site + YOB + Age + T/S	5	-77.352	168.915	6.399	0.011
Growth Rate ~ Site * YOB + Age	5	-77.507	169.224	6.708	0.009
Growth Rate ~ Site + YOB * Age	5	-77.827	169.864	7.348	0.007
Growth Rate ~ Site * YOB * T/S	8	-73.634	170.411	7.895	0.005
Growth Rate ~ Site * Age * T/S	6	-77.121	171.268	8.752	0.003
Growth Rate ~ Site * YOB * Age	6	-77.477	171.981	9.465	0.002
Growth Rate ~ Site * YOB * Age * T/S	12	-72.532	182.805	20.289	0.000