Causes and Consequences of Telomere Lengthening in a Wild Vertebrate Population

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Abstract

Telomeres have been advocated to be important markers of biological age in evolutionary and ecological studies. Telomeres usually shorten with age, and shortening is frequently associated with environmental stressors and increased subsequent mortality. Telomere lengthening – an apparent increase in telomere length between repeated samples from the same individual – also occurs. However, the exact circumstances, and consequences, of telomere lengthening are poorly understood. Using longitudinal data from the Seychelles warbler (Acrocephalus sechellensis), we tested whether telomere lengthening – which occurs in adults of this species – is associated with specific stressors (reproductive effort, food availability, malarial infection and cooperative breeding) and predicts subsequent survival. In females, telomere shortening was observed under greater stress (i.e. low food availability, malaria infection), while telomere lengthening was observed in females experiencing lower stress (i.e. high food availability, assisted by helpers, without malaria). The telomere dynamics of males were not associated with the key stressors tested. These results indicate that, at least for females, telomere lengthening occurs in circumstances more conducive to self-maintenance. Importantly, both females and males with lengthened telomeres had improved subsequent survival relative to individuals that displayed unchanged, or shortened, telomeres – indicating that telomere lengthening is associated with individual fitness. These results demonstrate that telomere dynamics are bidirectionally responsive to the level of stress that an individual faces, but may poorly reflect the accumulation of stress over the lifetime. This study challenges how we think of telomeres as a marker of biological age.

Introduction

Senescence – the deterioration of health and performance in old age – occurs in nearly all species (Nussey *et al.* , 2013). However, within species there can be considerable individual variation in the onset and rate of senescence (e.g. Lemaître *et al.* , 2013). Thus, individuals may be biologically older or younger than expected for their chronological age. Measuring 'biological age' (Baker and Sprott, 1988) is valuable, not only in regards to organismal health but also in terms of understanding fundamental concepts in ecology and evolution e.g. trade-offs in life-history strategies, or the impact of different environmental stressors (Stearns, 2008; Lemaître *et al.* , 2015).

Telomeres are repetitive nucleotide sequences at the ends of chromosomes, which protect the functional integrity of the genome. Due to the 'end replication problem' (Watson, 1972), telomeres shorten with each cell division, until a critical length is reached where cells can no longer divide (Olovnikov, 1996; Campisi,

2003) Telomeres also shorten when exposed to sources of cellular damage, such as reactive oxygen species (Von Zglinicki, 2002; Reichert and Stier, 2017). Both the number of cell-divisions and cell damage load are cumulative (i.e. age-dependent) but also variable in rate. Telomere length shortens with increasing age in a broad range of taxa (Barrett *et al.* , 2013; Bendix *et al.* , 2014; Stier *et al.* , 2015) but see Fairlie*et al.* (2016).

There is considerable empirical evidence to support the idea of telomere length being a marker of biological age. Accelerated telomere shortening occurs as an outcome of life-history or environmental conditions associated with increased cellular division and reactive oxygen species production, including developmental growth (Salomons *et al.* , 2009; Monaghan and Ozanne, 2018), early-life adversity (Boonekamp*et al.* , 2014; Watson, Bolton and Monaghan, 2015) and reproductive effort (Reichert *et al.* , 2014; Sudyka *et al.* , 2014). Shorter telomeres and/or higher attrition rates are associated with increased mortality risk (e.g. Haussmann, Winkler and Vleck, 2005; Vera*et al.* , 2012; Fairlie *et al.* , 2016; Barret *et al* ., 2013) and 'faster' life histories (Haussmann *et al.* , 2003; Sudyka, Arct, *et al.* , 2019). Therefore, telomere length has been proposed as a valuable biomarker linking past life-history costs to future performance (Young, 2018).

Over the last decade there has been a rapid expansion in studies investigating the causes and consequences of telomere dynamics across a wide range of taxa and environmental situations. However, our growing awareness of the complexity of telomere dynamics raises important questions on how we interpret telomeres as a biomarker. Longitudinal studies in humans and some wild vertebrates have shown that within-individual changes in telomere length are highly variable and bidirectional (Svenson *et al.* , 2011; Fairlie *et al.* , 2016; Hoelzl *et al.* , 2016; Spurgin *et al.* , 2017; van Lieshout*et al.* , 2019). Until recently, observations of telomere lengthening were often attributed to measurement error between samples collected too close in time – relative to the rate of telomere loss – to detect telomere shortening (Chen *et al.* , 2011; Steenstrup*et al.* , 2013). However, it is now recognised that the degree or frequency of observed telomere lengthening is often greater than that expected from measurement error alone (Bateson and Nettle, 2017; Spurgin*et al.* , 2017; van Lieshout *et al.* , 2019).

Telomere lengthening within the same individual may be observed for a variety of reasons. First, the enzyme telomerase can restore lost telomere length (Blackburn *et al.* , 1989). Since telomeres shorten during cell division, telomerase remains active in cell lineages requiring greater proliferation potential, such as haematopoietic stem cells (Morrison *et al.* , 1996; Haussmann *et al.* , 2007). Telomeres can also lengthen via alternative mechanisms, independent of telomerase (see Cesare and Reddel, 2010 for a discussion). Importantly, telomere measurements may increase in subsequent assays due to changes in clonal cell composition, i.e. an increase in long-telomere cells relative to short-telomere cells. All the mechanisms explained above are relevant to the telomere dynamics of blood, the tissue most often utilised for ecological and evolutionary studies on vertebrates (Nussey*et al.* , 2014). Furthermore, in mammals the proportions of circulating leucocyte cell types (with differing telomere lengths; Weng, 2012) can also change dramatically within an individual, for example in response to infection, resulting in apparent changes in overall telomere length (Beirne *et al.* , 2014). In birds and reptiles, blood-derived assays of telomere length overwhelmingly stem from nucleated erythrocytes (Stier *et al.* , 2015), and telomerase activation or turnover in haematopoietic cell lines could, in theory, create heterogeneity in measured telomere length.

The importance of (apparent) telomere lengthening in wild populations remains uncertain. Since telomere attrition occurs as a consequence of life-history or environmental stress costs, telomere lengthening may reflect investment in self-maintenance when those costs are alleviated. For example, wild edible dormice (*Glis glis*) that receive supplementary food showed lengthened telomeres (Hoelzl *et al.* , 2016). In other wild species, changes in telomere length reflected temporal differences in environmental conditions, with lengthening coinciding with more favourable environments (e.g. Mizutani *et al.* , 2013; Foley *et al.* , 2020). Telomere dynamics can also reflect changes in parasitic pressure. For example, infection with malaria has been associated with telomere attrition in wild and captive birds (Asghar *et al.* , 2016), but the clearing of infections in humans can result in lengthening (Asghar *et al.* , 2018). The ability of telomeres to both shorten and lengthen, rather than being an irreversible one-way ratchet, suggests that we may have to rethink our interpretation of telomere dynamics. Instead of reflecting the accumulation of all past stressors and growth,

telomere length may be more of a short-term marker, reflecting an individual's current condition consequent on the challenges and trade-offs faced by an individual. However, in contrast to telomere shortening, the circumstances under which telomere lengthening occurs in natural populations remain poorly understood.

Given the fitness costs associated with shorter, or more rapidly shortening, telomeres (see above), one might expect improved fitness to be associated with telomere lengthening. Recent reviews argue that telomere dynamics are a non-causal biomarker of accumulated cellular damage – such as that occurring from oxidative stress – that subsequently impacts fitness (Simons, 2015; Young, 2018). However, there is evidence that active restoration of telomere length can impact organismal performance. First, telomere lengthening could reduce the frequency of critically short telomeres – thought to directly contribute to organismal ageing by inducing cellular senescence and apoptosis (Vera *et al.* , 2012; Van Deursen, 2014). Secondly, telomerase has restorative effects on cells (Cong and Shay, 2008; Criscuolo *et al.* , 2018). Both telomerase activity and telomere lengthening are associated with tissue regeneration (Anchelin *et al.* , 2011; Reichert, Bize, *et al.* , 2014) and telomerase overexpression in mice is beneficial to a range of health parameters (Bernardes de Jesus *et al.* , 2012; Simons, 2015). Conversely, active telomere lengthening could also have negative effects, such as proliferating cancers (Shay and Wright, 2011) or by diverting energy from competing traits (Young, 2018). Nonetheless, telomere lengthening has the potential to be associated with organismal performance, and this impact is not dependent on telomere length playing a causal role in organismal ageing.

In this study, we aim to determine when and why telomere lengthening occurs, and assess its association with survival, in a wild population of the facultative cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*). Previous studies on this population have shown that telomeres shorten with age, and individuals with shorter telomeres are less likely to survive to the following year (Barrett *et al.* , 2013). Furthermore, telomere shortening is associated with various stresses in this species, including inbreeding (Bebbington *et al.* , 2016), intra-specific antagonistic interactions (Bebbington *et al.* , 2017) and parental care (Hammers *et al.* , 2019). However, telomere lengthening is often observed between successive samples taken from the same adult individual, and is greater than that expected from measurement error alone (Spurgin *et al.* , 2018). We predict that telomere lengthening occurs in individuals that experience reduced stress. Specifically, we predict that, for adults, telomere lengthening will be associated with reduced reproductive effort (less breeding, higher food availability and the presence of helpers) and an absence of malaria (the only known parasite in the population). We expect this relationship to be sex-specific, given that reproductive investments differ between sexes in this species (Hammers *et al.* , 2019; van Boheemen *et al.* , 2019). Furthermore, we tested whether increased survival is associated with telomere lengthening.

Methods

The Seychelles warbler model system

The Seychelles warbler is a small insectivorous passerine currently distributed across five islands in the Seychelles. The population on Cousin Island (29 ha; 4°20' S, 55°40' E) – containing *ca.* 320 individuals – has been extensively monitored since 1986 (Komdeur, 1992; Hammers *et al.* , 2015). Since 1997, nearly all individuals (>96%) have been ringed with a unique combination of a British Trust for Ornithology (BTO) metal ring and three colour rings for identification (Raj Pant *et al.* , 2020) (Richardson *et al.* , 2001). Individuals were usually first caught as nestlings, or as dependent juveniles (<8 months old) with mist nets (see Kingma *et al.* , 2016 for details). Juveniles were aged as fledglings (1–3 months), old fledglings (3–5 months) or sub-adults (5–12 months) based on behaviour and eye colour (Komdeur, 1992). Since the resighting probability of individuals during the major breeding season is close to one – 0.98 for individuals [?]2 years-old (Brouwer *et al.* , 2006) – and dispersal from the island is virtually absent (Komdeur *et al.* , 2004), individuals that were not observed during the major breeding season were assumed dead. First year survival is 0.61 +- 0.09 SE, increasing to 0.84 +- 0.04 SE annual survival in adults (Brouwer *et al.* , 2006). For individuals reaching fledgling age, the mean life expectancy is 5.5 years (Komdeur, 1991), and

the maximum recorded lifespan is 19 years (Hammers and Brouwer, 2017).

The population is structured into *ca* 115 clearly defined territories (Kingma *et al.* , 2016). The availability of the warbler's invertebrate prey (Komdeur, 1992) varies considerably due to the interacting effects of defoliating salt spray (along coastal territories), tree species abundance, elevation and rainfall (Van de Crommenacker *et al.* , 2011). Territories are defended year-round by a single dominant breeding pair, but *ca* 40% of territories include an additional 1–5 sexually mature subordinates, often past offspring of the same dominant pair (Richardson, Burke and Komdeur, 2002).

The majority of breeding activity (94% of territories) occurs from June to August, but a minor breeding season also occurs from January to March (Komdeur and Daan, 2005). Breeding attempts usually consist of one-egg clutches (Komdeur, 1994). Only females incubate while both sexes provision chicks and fledglings for *ca* three months post-fledging. Around one third of subordinates also provide alloparental care to group offspring, hereafter 'helpers' (Komdeur, 1994; Hammers *et al.* , 2019). About 44% of female helpers are also co-breeders (Richardson *et al.* , 2001; Raj Pant *et al.* , 2019). The offspring of cobreeders are jointly cared for by the subordinate female and dominant pair (Richardson *et al.* , 2001; Bebbington *et al.* , 2018). The frequency of extra-pair paternity in the population is high (*ca* 41%; Raj Pant *et al.* , 2019) and such paternity is nearly always gained by dominant males from other territories (Richardson *et al.* , 2001), but males only provide parental care in their own territory.

Data collection

Our study uses data collected from 1995 to 2015. Each year (June–September), during the major breeding season, each territory was visited at least every two weeks to determine the identity and status of group individuals. During visits, the dominant female was followed for at least 15 minutes to assess breeding activity (Richardson, Burke and Komdeur, 2007). Territories with an active nest were visited every 3–4 days until the nestling(s) have fledged or the breeding attempt failed. Observations of incubating and/or provisioning were used to estimate hatching/fledging dates, and to determine whether any subordinates present in the territory were helpers (Richardson, *et al* ., 2002; van Boheemen *et al.* , 2019). For each territory, the availability of food was calculated (following Komdeur, 1992). Briefly, the number of insects (on the undersides of leaves) was multiplied by the percentage cover of broad-leaf vegetation within territories. This number was then divided by the number of adult territory occupants to give food availability per individual (Brouwer *et al.* , 2006).

During each major breeding season, as much of the adult population as possible (normally around 30%) was caught and re-sampled: *ca* 25 ul of blood was taken from the brachial vein and stored in 100% ethanol (Richardson *et al.* , 2001). DNA extracted from the blood samples (following Richardson *et al.* , 2001) was used to confirm sex and assign parentage using MasterBayes 2.52 based on genotypes derived from 30 microsatellite loci (for details see Sparks *et al.* , 2020). The presence of haemosporidian infection (*Haemoproteus nucleocondensus* ; hereafter referred to as malaria) - the only known parasite in the Seychelles warbler (Hutchings, 2009) - was screened for following Hellgren *et al* . (2004). In the Seychelles warbler, nearly all individuals become infected with malaria in their first year (Hammers *et al.* , 2016). Thus, in our study (of individuals >1 year of age), birds in which we did not detect malaria may be in the latent infection stage – where parasites persist in organs - or have cleared infection, while infected individuals are either in the later chronic stage of infection, or in a subsequent relapse or reinfection. Infection with malaria does not appear to have an impact on annual survival in the Seychelles warbler (Hammers *et al.* , 2016) but has been linked to telomere attrition in the great reed warbler (*Acrocephalus arundinaceus* : Asghar *et al.* , 2015).

Relative Telomere Length (RTL; the concentration of amplified telomeric DNA relative to that amplified at *GAPDH* – a single copy gene) had previously been measured using qPCR as part of another study (Spurgin *et al.* , 2018). Since avian erythrocytes are nucleated and vastly outnumber other blood cell types,

blood RTL is effectively a measure of erythrocyte RTL (Stier *et al.* , 2015). Individuals with two or more RTL measurements were used in the current study, with the difference between consecutive pairs of RTL measurements (ΔRTL) as the response variable. We excluded RTL measurements from young individuals (< 1 year), as previous work in this population has shown that within-individual rate of attrition per annum is an order of magnitude greater in the first year compared to adult life (Spurgin *et al.* , 2018). For consistency, we focused on RTL measurements from catches only within the major breeding season, since inter-seasonal [?]RTL could reflect seasonal effects on RTL. Individuals are caught opportunistically, meaning that the follow-up period between RTL measurements (hereafter [?]RTL period) ranged from one year (i.e. consecutive seasons) to 9 years (Fig. S1). [?]RTL did not exhibit a significant relationship with the [?]RTL period. The final dataset comprised 359 ΔRTL measures from 227 adults.

Reproductive effort was measured as the number of offspring raised by an individual in the [?]RTL period; specifically, offspring that had hatched after time 1 and had reached independence (3 months old) before time 2. Social offspring – those for which a dominant breeder provides parental care – are determined from behavioral observations during nest attempts. Offspring are genotyped to identify genetic parentage. This is an underestimation of total offspring produced, since we excluded offspring for which parents could not be assigned (*ca* 15% of offspring) and some offspring are likely to have died before being sampled (Edwards, Burke and Dugdale, 2017). We used the number of social offspring as our estimate of reproductive output in males (since males do not care for offspring sired in other territories). Females (dominants or co-breeder) always contribute to the care of any offspring in the nest; (Richardson, Burke and Komdeur, 2002). Thus, female reproductive output was the number of co-bred offspring. For both sexes, the majority of individuals had 0–2 offspring within each [?]RTL period (Fig. S1). Offspring number was positively correlated with the [?]RTL period (Pearson's $r = 0.69$, $df = 357$, $P < 0.001$, Fig. 1S), meaning that individuals typically produced one offspring every two years.

During the [?]RTL period, we averaged food availability (insect abundance per individual per field season) across field seasons. Reproductive effort - in terms of time spent incubating and provisioning - of dominant breeders is reduced by the presence of helpers, including co-breeders (Hammers *et al.* , 2019; van Boheemen *et al.* , 2019). Reduced telomere attrition in dominant females has been associated with the presence of helpers in a previous study (Hammers*et al.* , 2019). Therefore, we determined whether nest helpers (including co-breeders) were present in the territories of individuals that produced offspring.

Statistical analysis

Using RStudio (v1.2.5033, Rstudio Team 2020) we tested whether food availability, reproductive output, helper presence and malaria status predicted [?]RTL, with the prediction that high food availability, low reproductive output, helper presence and no malaria infection - or a combination of these factors - would result in telomere lengthening. We adjusted [?]RTL following Verhulst *et al.* (2013a); this method subtracts the mean difference between successive samples expected from the regression-to-mean effect, estimated by the correlation between successive samples. In our dataset, this correlation was very weak (Peason's $r = 0.06$, $df = 357$ $P = 0.22$), as expected given the low within-individual consistency of RTL in this system (Spurgin *et al.* , 2018). This results in an adjusted [?]RTL (hereafter [?]RTL) which is equivalent to RTL at time 2; positive values indicate longer RTL and negative values indicate shorter RTL, relative to the population mean RTL. As expected, [?]RTL was strongly correlated with unadjusted [?]RTL (Pearson's $r = 0.71$, $df = 357$,$P < 0.001$), meaning that individuals with lengthened or shortened RTL (relative to their initial RTL) tended to have more positive or more negative [?]RTL, respectively.

The association between factors and [?]RTL was tested using Linear Mixed Models (package *lme4* v1.1-25; Bates *et al.* , 2015). We deliberately focused on a restricted set of fixed effects – chosen a priori based on logic and evidence of influencing telomere dynamics – to avoid data dredging, which could generate false-positive associations. Chosen fixed effects included mean food availability (continuous), number of offspring (continuous), malaria status (infected or uninfected), helper presence (yes or no). We also included age at time 1 (continuous), [?]RTL period and logical two-way interactions; for example, the effect of offspring

production on telomere maintenance may depend on food availability and/or helper presence. Effects were likely to differ between the sexes due to differing investments in reproduction, so to investigate sex-specific differences, while also avoiding the need for complex three-way interactions, separate models were created for males and females. We included the sample year of the first RTL measurement as a random factor. Variation in RTL between qPCR plates (Sparks *et al.* , 2020) could contribute to variation in [?]RTL, since RTL measurements from longitudinal samples were run on separate plates. Therefore, the plate identities of both RTL measurements per [?]RTL were included as random factors. Since individuals with three or more RTL measurements had multiple measures of [?]RTL, individual identity was also included as a random factor. Full models were reported after removing non-significant interactions. Offspring number, food availability and [?]RTL period were log10 transformed (for normality) and mean centered to remove collinearity between their main effects and interaction (Schielzeth, 2010). Since most individuals had 0–2 offspring, offspring number was reduced to a categorical variable (zero, one or [?]2) for graphical interpretation of interactions.

To test whether [?]RTL influenced subsequent survival, we performed a Cox proportional hazards regression analysis (package *survival*v3.2-7; Therneau, 2014). The response variable was the number of years an individual lived beyond the sampling date of its last RTL measurement. We included 16 individuals that were still alive in 2020 as right-censored data points. 13 individuals translocated to other islands post-sampling were excluded, leaving 214 individuals. Predictor variables were [?]RTL, sex and age at last RTL measurement (since older individuals are expected to have shorter remaining lifespans). Where multiple measures of [?]RTL were available per individual, we used only the last measurement, meaning that each [?]RTL value represents the last known change in telomere length before the individual died. We were interested in whether predicted hazard ratios exhibited a proportional change across the range of [?]RTL values, or whether the association was nonlinear. Therefore, we modelled [?]RTL as both a linear and quadratic function. In this model, positive hazard coefficients would indicate a decreased probability of survival with increasing values of the predictor variable. Hazard ratios represent the effect size of predictors; for example, a hazard ratio of 2 indicates that the risk of death is twice as high for the corresponding change in a predictor variable.

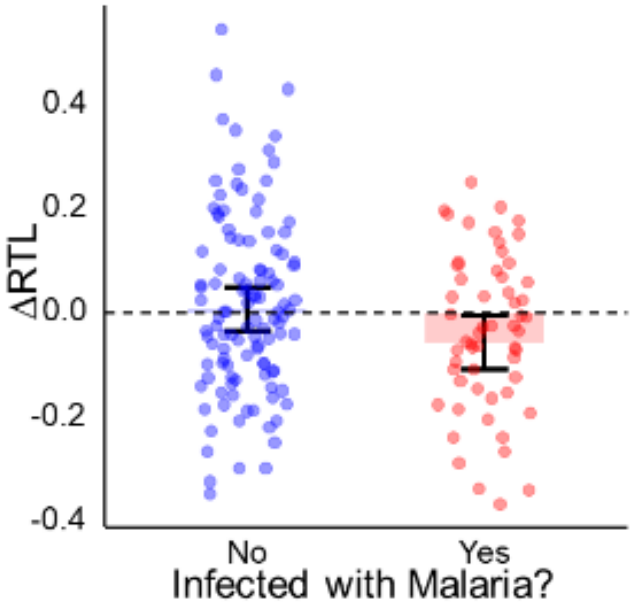
Results

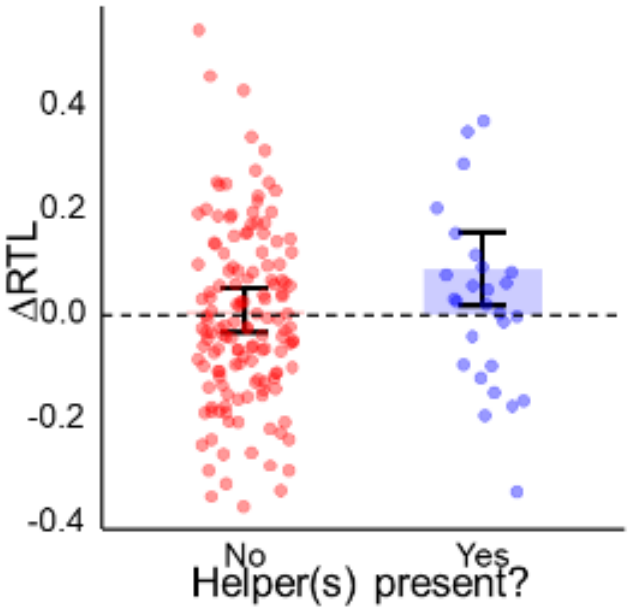
Telomere dynamics

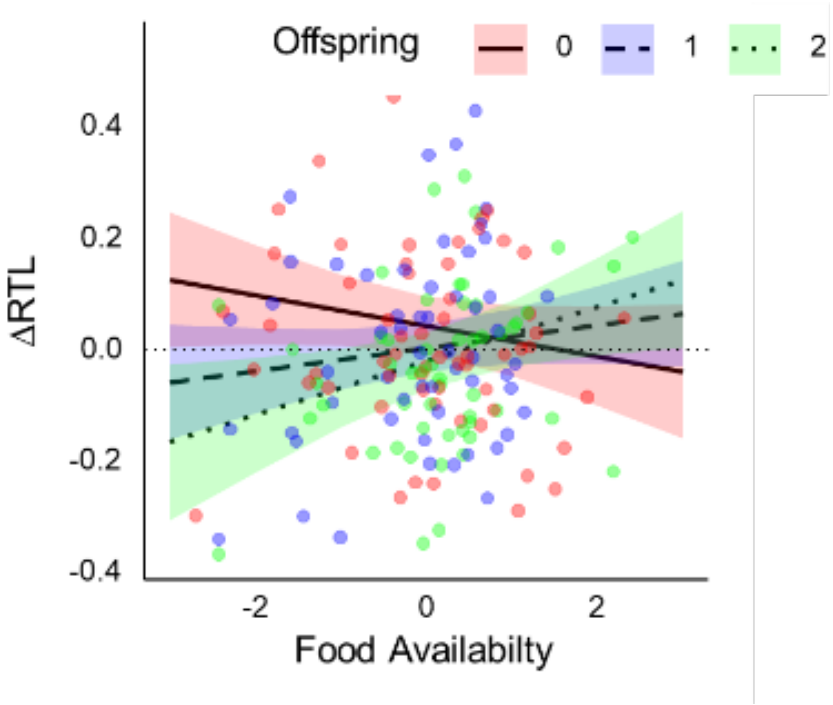
There was no difference in [?]RTL between males and females (Mean ± SE; Males = 0.001 ± 0.014, Females = 0.000 ± 0.013). Females infected with malaria at first RTL measurement had more negative [?]RTL than non-infected females (Fig. 1). In females, the association between offspring number and [?]RTL was dependent on the availability of food within the same period (interaction term in Table 1). Females that produced no offspring had [?]RTL close to zero regardless of food availability (Fig. 2). Females in territories of low mean food availability had a more negative [?]RTL with increasing numbers of offspring, whereas females in territories of high mean food availability had a more positive [?]RTL with increasing numbers of offspring (Fig. 2). Females that reared offspring with the assistance of helpers also had more positive [?]RTL change compared to females without helpers (Fig. 3). [?]RTL was not associated with [?]RTL period or age (Table 1). None of the chosen explanatory variables predicted [?]RTL in males (Table 1). In all models, results were qualitatively identical when unadjusted [?]RTL was used as the response variable and controlling for initial RTL length (Table S1).

FEMALES				
Predictor	**β**	**SE**	t	P
(Intercept)	0.036	0.032	1.119	0.266
Food Availability	0.017	0.015	1.127	0.263
Number of Offspring	**-0.033**	**0.016**	**-2.096**	**0.038**
Helper presence (Yes)	**0.080**	**0.037**	**2.163**	**0.032**

FEMALES				
Malaria (Yes)	**-0.064**	**0.027**	**-2.322**	**0.022**
[?]RTL period	-0.002	0.017	-0.126	0.900
Age	-0.007	0.006	-1.261	0.209
Food × Offspring	**0.040**	**0.014**	**2.893**	**0.004**
Random factors	**170 observations**	**Variance**		
Individual identity	108 individuals	< 0.001		
Catch year	18 years	0.001		
Plate 1 identity	54 plates	0.001		
Plate 2 identity	49 plates	0.002		
MALES				
Predictor	β	**SE**	t	P
(Intercept)	0.004	0.025	0.152	0.880
Food Availability	-0.002	0.015	-0.113	0.910
Number of Offspring	0.001	0.018	0.074	0.941
Helper presence (Yes)	0.016	0.039	0.394	0.694
Malaria (Yes)	-0.008	0.030	-0.286	0.775
[?]RTL period	-0.019	0.018	-1.035	0.303
Age	-0.015	0.015	-1.035	0.302
Random factors	**189 observations**	**Variance**		
Individual identity	119 individuals	0.006		
Catch year	16 years	0.001		
Plate 1 identity	65 plates	0.001		
Plate 2 identity	56 plates	0.005		





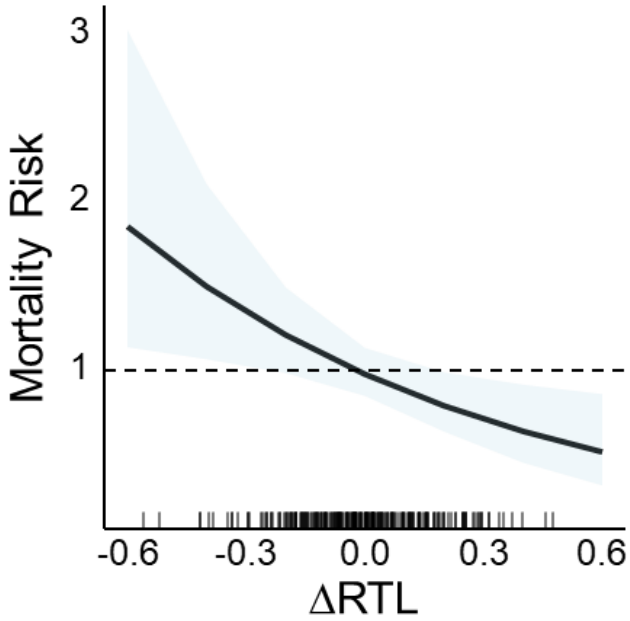


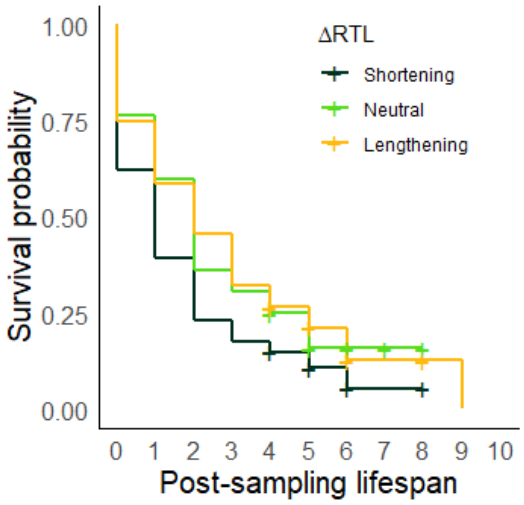
\protectTelomeredynamics and survival

Individuals with more negative [?]RTL values – indicating greater telomere shortening – had a greater subsequent risk of mortality (relative to individuals with no change in telomere length) while individuals with more positive [?]RTL values – indicating greater telomere lengthening – had a reduced risk of mortality (Fig. 4 & 5, Table 2). The quadratic function of [?]RTL was non-significant; thus, the effect of [?]RTL on

mortality risk was constant throughout the range of [?]RTL. The association between mortality risk and [?]RTL was not dependent on the sex or age of these adult individuals (interaction terms reentered into final model; Table 2). As expected, older individuals had an increased mortality risk. Visual inspection of Schoenfield residuals showed no violation of the assumption of non-proportional hazards, meaning the effects of predictor variables on mortality risk were constant throughout the remaining lifespan.

Predictor	β	**HR**	z	P
[?]RTL	**-1.067**	**0.344**	**-2.632**	**0.009**
[?]RTL^2	-0.217	0.805	-0.159	0.874
Sex (Male)	0.027	1.028	0.190	0.849
Age	**0.048**	**1.049**	**2.087**	**0.037**





Discussion

In our Seychelles warbler population, a change in telomere length ([?]RTL) was associated with life-history factors predicted to influence self-maintenance trade-offs - but only in females. Greater telomere shortening was observed in females with higher reproductive effort when living in areas of poorer food availability, as well as in individuals infected with malaria. Importantly, telomere lengthening was more often observed in females experiencing the reverse circumstance – i.e. those not infected with malaria and living in areas of high food availability – and those with helpers at the nest (the presence of which reduces reproductive effort per individual). These opposing changes in telomere length were not due to differences in initial telomere length or regression-to-the-mean effects. Consistent with [?]RTL being negatively correlated with stress, higher subsequent survival probabilities were associated with telomere lengthening, independently of sex and age.

We found that telomeres shortened in individuals that tested positive for malaria, but only in females. This finding is consistent with previous studies demonstrating greater erythrocyte telomere shortening in malaria-infected individuals compared to uninfected individuals (Asghar *et al.* , 2015; Karell *et al.* , 2017). While we expected malaria to affect both sexes equally, sex-specific differences in the impact of malaria on telomere length have also been observed in blue tits (*Cyanistes caeruleus* ; Sudyka, *et al.* , 2019). An emerging view is that telomere shortening is an outcome of immunological responses to infection (Giraudeau *et al.* , 2019). One such response – oxidative stress – is elevated in Seychelles warblers infected with malaria; albeit depending on the breeding stage (van de Crommenacker *et al.* , 2012). In our system, adults that test positive for malaria are in the chronic stage (i.e. late) and/or relapsed infections. Therefore, the telomere shortening we observed seems to reflect a cost of persistent infection – perhaps due to immunological responses - rather than a direct cost of parasitism, which tends to occur during the acute malarial stage (Asghar *et al.* , 2018). However, not knowing when malarial parasites became present or absent in blood, relative to the time of sampling, is a limitation of our observational study. Furthermore, while the frequency of reinfection/infection relapses is generally low (ca. 20% within two years; Hammers *et al.* , 2016), initially uninfected individuals could have undetected outbreaks within the time period of repeated samples.

We also found that telomere shortening was greater in females that produced more offspring. Moreover, the relationship only occurred when the mean food availability was low during the period of offspring production. Food limitation is expected to increase reproductive effort per unit of reproductive success, and thus increase the costs of reproduction (Harshman and Zera, 2007; Santos and Nakagawa, 2012). For example, individuals on poor-quality territories may have to work harder to meet the food demands of offspring, leading to elevated

stress (see Soulsbury and Halsey, 2018). Likewise, Seychelles warblers tend to be in poorer condition (in terms of oxidative stress and body mass) when provisioning chicks, compared to other nest stages, and when occupying poorer quality territories (Van de Crommenacker *et al.* , 2011, 2011). There are now several experimental and observational studies which show that individuals experiencing higher reproductive effort have shorter telomeres and/or experience greater telomere shortening (recently reviewed by Sudyka, 2019). However, few of these studies have explored associations between telomeres and reproduction in the context of food availability. Thus, our finding adds novel insight into life-history framework of telomere dynamics.

The relationship between telomeres and reproductive effort was only apparent in females. This was expected, since parental effort is higher in females; in the Seychelles warbler only females incubate and they also have higher provisioning rates than males (Hammers *et al.* , 2019; van Boheemen *et al.* , 2019). Thus, females benefit more from having nest helpers (Hammers *et al.* , 2019) and may be more responsive to differences in food availability when caring for offspring (e.g. Low *et al.* , 2012). Alternatively, telomere shortening may correlate with egg production – which is associated with substantial self-maintenance costs (Visser and Lessells, 2001; Williams, 2005) – more than with provisioning effort. Likewise, male telomere shortening may be more correlated with male-specific reproductive behaviours that were not accounted for in this study. For example, Bebbington *et al.* (2017) showed that Seychelles warbler males, which are more involved in territory defence, have more telomere shortening with increased competition from rival males.

Telomere length increased in females that produced more offspring when experiencing higher food availability, and when the production of offspring was assisted by nest helpers. This finding supports our main prior prediction – that telomere lengthening occurs in individuals experiencing lower levels of life-history stress. The non-biological explanation – that observed telomere lengthening is a consequence of high measurement error relative to attrition rate (Steenstrup *et al.* , 2013) – seems unlikely for several reasons. First, the degree of within-individual telomere lengthening observed in our system is greater than that expected from measurement error alone (Spurgin *et al.* , 2018). Secondly, high measurement error and a lack of telomere shortening would result in no overall change (i.e. a random scatter of values around zero), whereas we observed an overall increase in telomere length consistent with our predictions. Lastly, our analysis accounted for regression-to-the-mean effects. This suggests that 'real' telomere lengthening (i.e. that which is not purely a consequence of measurement error) is more frequent in individuals with less stressful life-histories.

Telomere lengthening may be an outcome of lower reproductive costs associated with high food availability, cooperative breeding and absence of parasite infection. As discussed above, reduced reproductive effort is associated with the maintenance of longer telomeres and/or reduced telomere attrition. Some experimental studies that manipulated offspring number find no change or even slight telomere lengthening in treatment groups with the fewest offspring (Kotrschal, Ilmonen and Penn, 2007; Heidinger *et al.* , 2012; Sudyka *et al.* , 2014); however, these observations tend to be reported as reduced telomere shortening. Furthermore, reduced oxidative damage and telomere lengthening have also been observed in wild rodents receiving food supplements (Fletcher*et al.* , 2013; Hoelzl *et al.* , 2016). Thus, telomere lengthening may occur because plentiful food permits the allocation of energy to mechanisms involved in restoring previously lost telomere length, such as telomerase. This is a possibility in the Seychelles warbler, as high telomerase activity has been observed in the bone marrow (relative to other tissues) in adults of other bird species (Haussmann *et al.* , 2007). Alternatively, telomere lengthening may be associated with the more successful life-histories of higher-quality females, rather than a consequence of low life-history stress (Bauch, Becker and Verhulst, 2013; Angelier *et al.* , 2019). Nevertheless, our study indicates that the telomere lengthening observed in some wild populations is not necessarily random or merely an artefact of measurement error, but can instead be associated with important life-history traits and/or trade-offs.

We found that individuals with greater telomere shortening had lower survival prospects. This finding is consistent with short telomeres being negatively associated with survival in the Seychelles warbler (Barrett *et al.* , 2013) and in a range other wild vertebrate species (reviewed in Wilbourn *et al.* , 2018). Telomere shortening can directly impact survival by increasing the frequency of critically short telomeres, which can trigger cellular senescence (Kurz *et al.* , 2004). However, our measure of telomere length is a mean value (i.e.

across chromosomes and cells) rather than a measure of the frequency of short telomeres *per se* (Bendix *et al.* , 2010). The non-causal explanation is that factors which shorten telomeres – such as oxidative stress – also cause wider cellular damage that ultimately increases mortality risk. Interestingly, we show that infection with malaria, for which we have not been able to find a survival impact in this species (Hammers *et al.* , 2016), may increase mortality risk via mechanisms that also shorten telomeres. Importantly, the telomere–survival relationship was not solely driven by negative effects (i.e. cellular damage and/or critically short telomeres), since individuals with lengthened telomeres had better survival prospects relative to individuals with no change in telomere length. This finding is consistent with the positive health and longevity effects of telomerase in mice (Bernardes de Jesus *et al.* , 2012; Simons, 2015) but contrasts with the results of Wood and Young, (2019), who found that increased telomere length was not associated with higher nestling survival in white-browed sparrow-weavers (*Plocepasser mahali*). In our study, telomere lengthening was also associated with life-history traits known to benefit survival and longevity in the Seychelles warbler: high food availability (Brouwer*et al.* , 2006) and helpers (Hammers *et al.* , 2019). This suggests that telomere lengthening may be characteristic of a strategy in which individuals make higher reproductive investments in more favourable environments, without incurring survival costs (as suggested by Hoelzl *et al.* , 2016).

Our study adds to the growing body of literature on the bidirectionality of within-individual telomere dynamics in ecological settings. We found that telomere lengthening can reflect good current environmental conditions and subsequently is linked to better survival prospects. Therefore, single measures of telomere length may not be a reliable indicator of damage accumulated in an individual's past life, nor their future performance – and hence not a good biological age marker. Future studies should determine the mechanisms behind telomere lengthening observed in wild populations, and whether telomere lengthening is coordinated across multiple tissue types within individuals.

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Authors contributions

The study was conceived by T.J.B and D.S.R. Data was collected by D.S.R, H.L.D, L.S, T.B and J.K. Statistical analyses were conducted by T.J.B with input from D.S.R and H.L.D. The paper was written by T.J.B and all authors critiqued the output with important intellectual content. All authors gave final approval for publication.

Competing Interests Statement

We report no competing interests

Ethics statement

All fieldwork was conducted in accordance with local ethical regulations and agreements. The Seychelles Bureau of Standards and Department of Environment gave permission for sampling and fieldwork. Nature Seychelles gave permission to carry out research on Cousin Island.

Data accessibility statement

Data files and code will be made available from the Dryad Digital Repository

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