**Spatiotemporal variation in lifelong telomere dynamics in the Seychelles warbler**

Lewis G. Spurgin1, Kat Bebbington1, Eleanor A. Fairfield1, Martijn Hammers2, Jan Komdeur2, Terry Burke3, Hannah, L. Dugdale2,4, and David S. Richardson1,5,.

1. School of Biological Sciences, University of East Anglia, Norwich Research Park, NR4 7TJ, United Kingdom
2. Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands
3. Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
4. School of Biology, The Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK
5. Nature Seychelles, Roche Caiman, Mahé, Republic of Seychelles

**Correspondence:** Lewis Spurgin: [l.spurgin@uea.ac.uk](mailto:l.spurgin@uea.ac.uk); David Richardson: [david.richardson@uea.ac.uk](mailto:david.richardson@uea.ac.uk)

**Running head:** Early-life telomeres

### Abstract

1. Understanding the costs individuals and populations face throughout their lifetimes is fundamental to understanding life-history evolution and population dynamics. Telomeres, the protective caps at the ends of chromosomes, shorten in response to oxidative stress, and telomere shortening is correlated with reduced survival. Thus, telomere dynamics may help us quantify individual variation in costs, and enhance our understanding individual variation in natural populations.
2. The Seychelles warbler (*Acrocephalus sechellensis*) is an excellent model system for disentangling the causes and consequences of individual variation in telomere dynamics. Here we study spatiotemporal variation in lifelong telomere dynamics in the Seychelles warbler. We combine long-term ecological data with one the largest longitudinal telomere datasets to date, consisting of 1808 samples from 22 cohorts born between 1993 and 2014. We provide a detailed analysis of how telomere dynamics vary over individual lifespans, and with spatiotemporal variation in the social and ecological environment.
3. We found that telomere length decreases with cross-sectional and longitudinal measures of age, and that telomere length decreases most rapidly early in life. However, both cross-sectional and longitudinal data suggested that telomere length increases occurs in some individuals for short periods, and using a large number of repeated measurements we show statistically that this increase cannot be explained solely by qPCR measurement error.
4. Telomere length and rates of shortening varied markedly both within and among cohorts, and cohort-level differences in telomere length persisted throughout individual lifespan. Temporal variation in telomere length was positively associated with island-wide temporal variation in food availability. The other significant predictors of telomere length were sex, with males having longer telomeres than females, and tarus length, which affected telomere length in a sex-specific manner.
5. Our comprehensive, long-term data show that in natural populations, telomere dynamics vary enormously over both space and time. Ascertaining what explains this variation requires combining large sample sizes spanning multiple cohorts, and long-term ecological data.

**Keywords:** Life-history; Telomere; Seychelles warbler; Senescence

**Data archival location:** This manuscript was written in R Markdown (<http://rmarkdown.rstudio.com/>). All data and scripts required to reproduce the manuscript, figures and analyses will be made available on GitHub.

PAGEBREAK

### Introduction

Telomeres shorten with age (Monaghan and Haussmann 2006), and in response to oxidative stress, which can be elevated due to environmental factors (Von Zglinicki 2002). When telomeres become critically short cells senesce (Campisi 2003), and the accumulation of these cells can result in organismal senescence and death (Wong et al. 2003). This association between senescence and telomere length has inspired a great deal of recent research into telomere evolutionary ecology (reviewed in Horn et al. 2010; Haussmann and Marchetto 2010; Monaghan 2014). While there is little direct evidence that the relationship between telomere dynamics and survival is causal (Simons 2015), there is mounting evidence that telomeres can act as biomarkers of cost in wild populations, providing a signature of the ecological stress that has been experienced and is otherwise difficult to detect (Monaghan 2014; Schultner et al. 2014; Asghar et al. 2015).

There is evidence from a range of taxa that the greatest rate of telomere shortening occurs in early life (e.g. Frenck et al. 1998; Haussmann et al. 2003), and that the extent of this shortening is influenced by the conditions experienced during that period (Price et al. 2013; Monaghan 2014; Nettle et al. 2015b; Reichert et al. 2015). Early-life telomere dynamics have been associated with both short-term and late-life survival (Heidinger et al. 2012; Boonekamp et al. 2014), and with other parameters such as cognition (Nettle et al. 2015a). However, few studies have simultaneously analysed how telomere dynamics, early-life conditions and late-life survival are all related in a natural setting. Moreover, how early-life telomere dynamics vary over spatial and temporal scales is poorly understood.

The longitudinal study (since 1986) of the Seychelles warbler (*Acrocephalus sechellensis*) population on Cousin Island provides an excellent system for studying senescence in the wild (reviewed in Hammers et al. 2015). Due to the isolated nature of the study population and intensive field monitoring, we have unusually comprehensive survival data and tissue samples spanning many years (see Methods, below). Ecological conditions and warbler population density on Cousin vary across space and time due to weather-induced changes in foliage cover and food availability (Van de Crommenacker et al. 2011). Seychelles warblers remain on their natal territories for at least six months, and variation in the oxidative stress experienced by individuals is associated with natal territory quality (Van de Crommenacker et al. 2011). However, neither early-life nor adult survival appear to be associated with natal territory quality or natal local density (Brouwer et al. 2006; Hammers et al. 2013). Facultative cooperative breeding occurs in the Seychelles warbler (Komdeur 1994; Richardson et al. 2003b), and the presence of helping subordinates (but not non-helping subordinates) in the natal territory is associated with increased survival later in life (Brouwer et al. 2012). Lastly, we have an established protocol for assessing telomere length in this species (**???**; Barrett et al. 2012), and telomere length predicts survival independently of age in adult Seychelles warblers, suggesting that telomeres act as a biomarker of cost in this species (Barrett et al. 2013). Thus, we have an excellent system in which to assess the costs of different social and environmental conditions experienced early in life, and to assess the later-life consequences of early-life conditions.

In this study we examine how telomeres are related to early-life environmental variation over the entire lifespans across multiple cohorts of the Seychelles warbler.

### Methods

##### Study species and sampling

The Seychelles warbler is a small (~15 g), insectivorous passerine bird with a mean life expectancy of 5.5 years at fledging (Hammers et al. 2013). The population of *ca*. 320 birds on Cousin Island (04'20'S, 55'40'E) has been intensively studied since 1986 (Richardson et al. 2003a; Spurgin et al. 2014). This species' main breeding season runs from June-August, when the breeding females on each of the *ca*. 115 territories lay one or, rarely, two or three eggs (Komdeur et al. 1991). As a result of this low reproductive output, combined with higher mortality in first-year birds (39%; Brouwer et al. 2006), cohort sizes in the Seychelles warbler are small (< 50).

Individuals are usually ringed in their first year of life, and so are of known age. They are then followed throughout their lives, and as they are non-migratory endemics naturally confined to the island (Komdeur et al. 2004), a biannual census of birds on Cousin during each breeding season gives accurate measures of local density, social status (e.g. breeding male/female, helping subordinate, non-helping subordinate) and individual survival (Crommenacker et al. 2011; Barrett et al. 2013). The isolated nature of the Cousin population is a key advantage of the system for analyses involving survival, which in other systems are often confounded by emigration (see Ergon and Gardner 2014 for a recent discussion). Full details of catching and monitoring methods can be found in Brouwer *et al.* (2012).

Seychelles warblers are highly territorial and all territories are mapped during the breeding seasons using detailed observational data of foraging and territorial defence behaviour, and surveyed for territory quality (Richardson et al. 2003a). Territory quality is calculated based on territory size, foliage cover and insect abundance (Komdeur 1992), and where territory quality estimates were not available for a specific year we used the average value for that territory across years (Hammers et al. 2013). Cousin is subject to intra- and inter-annual variation in rainfall and food availability, and such island-wide temporal variation may override the effects of individual territory quality. As an estimate of seasonal variation in food availability, we calculated an index of the number of insects across the entire island during each breeding season. This index is calculated as the mean number of insects found per unit leaf area over all surveys carried out on the island in a breeding season.

Each time a bird is caught on Cousin body mass and tarsus length are measured (to the nearest 0.1g and 0.1mm, respectively). Using information on eye colour (Komdeur 1991) and previous captures (Richardson et al. 2003a), we grouped birds into three age categories: one month old (birds still in the nest), 6 months old (fledglings with light grey eyes) and 10 months old (subadults with light brown eyes). A blood sample (*ca* 25 l) is taken from each bird captured via brachial venipuncture, and stored at room temperature in 1 ml of absolute ethanol in a 1.5 ml screw-cap microfuge tube.

##### Molecular methods

For each sample, genomic DNA was extracted from a ~2 mm2 flake of preserved blood using the DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer's protocol, with the modification of overnight lysis at 37oC and a final DNA elution volume of 80 l. Sex was determined using the PCR-based method outlined by Griffiths *et al.* (1998). Prior to telomere analysis, DNA concentration and purity were quantified using a NanoDrop 8000 Spectrophotometer (ThermoScientific), and the following thresholds were applied before samples were included for further analysis: i) DNA concentration must be at least 15 ng l-1 (based on a mean of three measurements), ii) the 260/280 ratio has to be between 1.8 and 2 and, iii) the 260/230 ratio should be higher than 1.8. DNA integrity was further validated by visualization with ethidium bromide after electrophoresis on a 1.2% agarose gel, and all samples with evidence of DNA degradation were re-extracted or excluded. All DNA extractions that passed the above criteria were diluted to 3.3 ng l-1 before telomere measurement. We measured relative telomere length (RTL) for all samples using a quantitative PCR (qPCR) assay of telomeres and a GAPDH control gene, using the molecular methods outlined by Bebbington *et al.* (**???**). We assessed repeatability of RTL using the rptR package in R (R Development Core Team 2011).

For a subset of birds we had longitudinal data, with an additional samples taken at different ages. For these individuals we calculated the within-individual change in RTL by subtracting RTL at time point *t* from RTL at timepoint *t* + 1 (hereafter RTL). Negative values of RTL reflect telomere shortening, while positive values reflect telomere lengthening.

##### Statistical analyses

We performed all statistical analyses using R. We first explored the relationship between RTL and age among cohorts using linear mixed models (LMMs) carried out in the lme4 package (**???**). Following a similar approach to Fairlie *et al.* (**???**), we compared a selection of models fitting different relationships between RTL and age. We created models where age was fitted as a linear covariate, where age was log-transformed, and where age was fitted as a factor. We also created models including birth year (cohort) as a factor, and ones which included interactions between cohort and each measure of age. All fitted models are included in Table 1. As random effects we included individual ID, catch year and qPCR plate ID. Models were compared using AIC with correction for finite sample size (AICc). Using the longitudinal data, we tested whether telomores shorten with age in individuals, using LMMs of RTL and age (a longitudinal measure based on within-subject centring; Pol and Wright 2009). We calculated age using log and polynomial transformed age data, and carried out model selection as above, with the exception that we did not model age as a factor (due to a lack of discreet groupings).

Because, when examining the distribution of RTL, we observed apparent telomere lengthening in our data, we used the entire Seychelles warbler dataset to test whether this lengthening could be explained by measurement error. Using all birds (adults and juveniles) with at least two telomere measurement, we calculated RTL for each pair of samples (for example, for a bird with three longitudinal measurements throughout their life we calculated the change in RTL between the first and second sample and between the second and third sample). We then compared these longitudinal changes in telomere length to sampling error, using all samples where we had at least two repeat telomere measurements for the same sample (i.e. completely seperate reactions run on separate plates: N = 422 measurements from 293 samples). We calculated RTL between pairs of repeat measurements within samples in exactly the same way as for across samples, except that repeat measurements were ordered by the date at the qPCR was run rather than by age. We first compared the variance in RTL within and among individuals using a Levene's test. Then, to separately test whether the extent of telomere shortening and lenghtening within individuals was greater than expected by measurement error, we split RTL measurements into groups in which shortening (RTL < 0) and lengthening RTL (RTL > 0) occurred, and tested whether RTL among samples was significantly different between within-sample and across-sample measurements, using Wilcoxon tests.

We used LMMs to test how variation in early-life environmental and social conditions influenced telomere length within cohorts. We created a full model with RTL as a response variable, alongside the following explanatory variables: age, tarsus length, body mass, sex, annual food availability, territory quality, population density (an annual measure estimated from the summer breeding census) and the number of birds and the number of helping subordinate birds present in the territory. As random effects we included catch year and individual identity. We report model estimates and confidence intervals for all effects included in the full model. To obtain an estimate of explanatory power, we calculated marginal R^2 (just incoporatng fixed effects) and conditional R^2 (incorporating fixed and random effects) following Nakagawa & Schielzeth (**???**) and Johnson (**???**), respectively As a complementary approach, we also performed model averaging, using the MuMIn package in R (Bartoń 2012). Model selection was performed using the full model described above and a top model set defined, containing all models with AICc 6 compared to the best supported model (Burnham et al. 2011). We report model-averaged coefficients, confidence intervals and 'relative importance', which reflects the relative weights of each predictor variable across the top model set. For individuals with longitudinal data (*n* = 655) we repeated the above analyses of telomere dynamics, replacing telomere length with RTL as the response variable, and excluding the plate ID random effect (as each measurement of RTL was based on two or more measurements, and so run on multiple plates).

### Results

We measured telomere lengths using a total of 1808 unique samples from juvenile and adult Seychelles warblers from 22 cohorts born between 1993 and 2014. Efficiencies (mean s.d.) for our telomere and GAPDH reactions were (1.78 0.05) and (1.92 0.04) respectively. Inter-plate repeatability of RTL, based on 422 samples measured at least twice, was 0.68 (CI = 0.64-0.71).

##### Telomere dynamics and age amnong cohorts

We first tested how RTL was related to age among cohorts using a model selection approach. We found that the top model, by some distance, contained a loglinear relationship between RTL and age, as well as cohort ID and a (log) age x cohort interaction (Table 1A). The second best model contained log age and cohort ID, with no interaction term, although this was substantially poorer in terms of model fit (AICc > 10), and all other models were much poorer still (AICc > 35; Table 1). RTL decreased with log age (estimate = -0.050, CIs = -0.064, -0.036), with the greatest decrease occurring early in life (Fig. 1A). There was also substantial variation in RTL among cohorts (Fig. 1B). There was a negative relationship between RTL and age in almost all of the 22 cohorts, but in accordance with an age x cohort interaction, the slope of the relationship between log age and RTL varied substantially (Fig.1C).

An within-individual analysis of age revealed that the top model explaining RTL contained log age, which reflects within-individual changes in log-transformed age (Table 1B). Models including cohort ID and cohort x age interactions were substantially poorer than models only containing age (Table 1B). RTL dereased with log age (estimate = -0.052, CIs = -0.085, -0.018), confirming that within-individual telomere shortening occurs across the Seychelles warbler dataset.

Within individuals, longitudinal data showed that there was a slight positive correlation between telomere measurements taken from different timepoints (Fig. 2A), but this was weak (marginal R2 = 0.01), and not quite significant (estimate = NA, CIs = -0.002, 0.138). Although both or cross-sectional and longitudinal data indicated a general trend of telomere shortening with age, we found that 44% of our RTL measurments were positive, and thus indicative of telomere lengthening. To test whether the apparent telomere lengthening in our dataset could be explained by measurement error, we compared variance in telomere length among repeat measurements of samples to the variance observed among different samples of the same individual (see methods for details). We found significantly higher variance in telomere length over individual lifetimes compared to among sample replicates (Levene's test: F = 43.63; P < 0.001; Fig. 2B). Importantly, splitting the longitudinal data into instances of shortening (i.e. RTL < 0) and lenghtning (i.e. RTL > 0) revealed that not only did we observe significantly more shortening within individuals compared to within samples (Wilcoxon test: W = 3.455810^{4}; P < 0.001), but also significantly more lengthening (W = 31123; P < 0.001; Fig. 2B).

##### Telomere dynamics and the environment

A LMM revealed that age, tarsus length, sex and annual variation in food availability were significantly related to RTL (Table 1). Tarsus length was negatively related to RTL and males had longer telomeres than females (Table 1; Fig. 4A), while insect availability was positively related to RTL (Table 1; Fig. 4B). The full model was weak in terms of explanatory power of fixed effects (marginal R2 = 0.07), although including the random effect terms increased this substantially (conditional R2 = 0.26) The model averaging approach yielded qualitatively identical results to the full LMM, with the same explanatory variables 'significant' in terms of being retained in top models, and having model-averaged confidence intervals not overlapping zero (Table S1; Fig. S1). One interesting finding from the model selection was that sex only appeared in top models where tarsus length was also present (Table S1). In accordance with this, when tarsus length was removed from the full model sex was no longer significant (estimate = 0.012, CIs = -0.010, 0.034), and a sex x tarsus interaction was significant when included (estimate = 0.025, CIs = 0.006, 0.043); RTL increased with tarsus length in males, but decreased in females (Fig. 4A). No social or ecological environmental variables were significant predictors of RTL, regardless of whether we interpreted the full model (Table 2), or used model averaging (Table S2; Fig. S2).

### Discussion

Here we use a long-term, multi-cohort dataset to assess the relationships between the spatiotemporal variation in the ecological environment and lifelong telomere dynamics in a closed population of Seychelles warblers. We found that telomeres shorten over time, and that telomere loss is greatest in early life. However, bouts of apparent telomere lengthening occur, and the extent of this lengthening cannot be explained solely by qPCR measurement error. We also found that telomere dynamics vary among sexes and over time, with temporal variation in telomere dynamics related to fluctuations in food availability.

Our study adds the the now substantial body of literature from humans and wild animals, showing that telomere length decreases with age, and that this decrease is most rapid in early life (**???**; e.g. Frenck et al. 1998; Haussmann et al. 2003; Heidinger et al. 2012). However, using longitudinal data we found that within-individual telomere measurments were only weakly correlated, and that consistent telomere shortening only occurs early in life, after which both shortening and lengthening occurs. This is in contrast to other avian studies where birds were reared in laboratory (Heidinger et al. 2012) or nest-box (Boonekamp et al. 2014) conditions, in which juvenile and adult telomere length were highly correlated, and individual-level telomere shortening clearly occurs throughout the juvenile period, and into adulthood. However, in accordance with our study, a recent study on a wild population of Soay sheep (*Ovis aries*) found only a very weak correlation between juvenile and adult RTL, and a highly complex pattern of telomere dynamics across the lifespan (**???**).

We also found evidence for sex-specific telomere dynamics in Seychelles warblers, with males having longer-telomeres than females, in and that this sex-difference interacts with tarsus length. This is likely to be due to differential growth costs between sexes. Furthermore, in Seychelles warblers the effect of telomere length on survival is strongest for males (Barrett et al. 2013). Together, these results suggest that sex effects should be considered when studying any aspect of telomere dynamics (see also Barrett and Richardson 2011).

Perhaps the clearest result from our study is that RTL, among cohorts, and among sampling years. A few studies have now shown that temporal variation in telomere dynamics occurs in natural populations, although these have been limited in the number of seasons (**???**; Mizutani et al. 2013; Watson et al. 2015). The long-term Seychelles warbler dataset has allowed us to show that temporal variation in telomere dynamics can occur over substantial time periods. Moreover, because of the large number of cohorts we have been able to show statistically that that temporal variation in RTL is related to variation in food availability (Fig. 4B). If cohort-level variation in telomere dynamics continues to be found in other systems, this has potential consequences for our understanding of telomere dynamics in natural populations. In particular, our findings suggest that the telomere dynamics of a population at a given point in time represent a snapshot of a temporally varying process. More research is now needed within and across multiple cohorts and populations to better understand how how and why population-level telomere dynamics vary over space and time.

Despite substantial cohort-level variation in RTL, within cohorts we found no evidence that any of the social or environmental variables we measured affect juvenile RTL. This is surprising, as a poor social and ecological environment is known to be detrimental to juvenile Seychelles warblers, both in terms of oxidative stress and later life survival (Van de Crommenacker et al. 2011; Brouwer et al. 2012). An explanation for this could be that temporal variation in RTL in our data masks our ability to detect spatial trends. However, this is unlikely as we controlled for cohort ID, and within-cohort variation in RTL greatly exceeds among-cohort variation (Fig. 1C). A second possibility is that effects are generally weak and levels of noise in our telomere measurements and/or ecological data preclude detection of significant effects. While noise in telomere measurements and problems with power are a problem in any study using qPCR to measure telomere length (Nussey et al. 2014), our sample sizes and levels of repeatability compare favourably with other studies of natural populations that have found significant effects of the early-life environment on telomere length (e.g. **???**). More likely is that early-life RTL in the Seychelles warbler is explained by a set of variables that we have not measured. A key question to be addressed is the extent to which early-life RTL reflects inheritance (Asghar et al. 2014; e.g. Becker et al. 2015). This is important, as inheritance may capture a large amount of unexplained variation in early-life RTL within our dataset. Future research on the Seychelles warbler will examine, in a quantitative genetic framework, how genetic and environmental components, and their interactions, affect telomere dynamics and senescence.

While the relationship between mortality and telomeres in adults has been established for some time (Cawthon et al. 2003), only recently has the link between later-life survival and early-life telomere dynamics been studied. In captive zebra finches, juvenile telomere length predicts late-life survival (Heidinger et al. 2012), and in wild bird populations survival to the nestling phase (Watson et al. 2015), and survival to adulthood (Boonekamp et al. 2014), have been linked with early-life telomere dynamics. It is worth noting that here we have considered survival, but telomere length and shortening in early life may also be linked to other components of fitness (reviewed in Monaghan 2014). Reproductive senescence occurs in the Seychelles warbler (reviewed in Hammers et al. 2015), making this system well suited to examining how telomere length predicts lifetime reproductive success. We expect that by gaining a fuller understanding of telomere dynamics in natural populations, the fields of life-history evolution and evolutionary ecology will be greatly enhanced.

### Acknowledgements

We thank Nature Seychelles for facilitating the long-term Seychelles warbler project. The Seychelles Bureau of Standards and Department of Environment gave permission for sampling and fieldwork. Emma Barrett laid the foundations for this study, generating the original telomere qPCR protocol. We thank everyone who has helped in the field, and the Seychelles warbler research group for discussions. This work was funded by two Natural Environment Research Council (NERC) grants to DSR (NE/F02083X/1 and NE/K005502/1). LGS was also funded by a fellowship from the BBSRC, and HLD by a NERC fellowship.

### References

Asghar, M., S. Bensch, M. Tarka, B. Hansson, and D. Hasselquist. 2014. Maternal and genetic factors determine early life telomere length. Proceedings of the Royal Society B: Biological Sciences 282:20142263–20142263.

Asghar, M., D. Hasselquist, B. Hansson, P. Zehtindjiev, H. Westerdahl, and S. Bensch. 2015. Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. Science 347:436–438.

Barrett, E. L. B., and D. S. Richardson. 2011. Sex differences in telomeres and lifespan. Aging Cell 10:913–21.

Barrett, E. L. B., W. Boner, E. Mulder, P. Monaghan, S. Verhulst, and D. S. Richardson. 2012. Absolute standards as a useful addition to the avian quantitative PCR telomere assay. Journal of Avian Biology 43:571–576.

Barrett, E. L. B., T. Burke, M. Hammers, J. Komdeur, and D. S. Richardson. 2013. Telomere length and dynamics predict mortality in a wild longitudinal study. Molecular Ecology 22:249–259.

Bartoń, K. 2012. Package ‘MuMIn’. Model selection and model averaging base on information criteria. R package version 1.7.11.

Becker, P. J. J., S. Reichert, S. Zahn, J. Hegelbach, S. Massemin, L. F. Keller, E. Postma, and F. Criscuolo. 2015. Mother-offspring and nest-mate resemblance but no heritability in early-life telomere length in white-throated dippers. Proceedings of the Royal Society B: Biological Sciences 282:20142924.

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

Brouwer, L., D. S. Richardson, C. Eikenaar, and J. Komdeur. 2006. The role of group size and environmental factors on survival in a cooperatively breeding tropical passerine. Journal of Animal Ecology 75:1321–1329.

Brouwer, L., D. Richardson, and J. Komdeur. 2012. Helpers at the nest improve late-life offspring performance: evidence from a long-term study and a cross-foster experiment. PLoS ONE 7:e33167.

Burnham, K., D. Anderson, and K. Huyvaert. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. Behavioral Ecology and Sociobiology 65:23–25.

Campisi, J. 2003. Cellular senescence and apoptosis: How cellular responses might influence aging phenotypes. Experimental Gerontology 38:5–11.

Cawthon, R. M., K. R. Smith, E. O’Brien, A. Sivatchenko, and R. A. Kerber. 2003. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 361:393–395.

Crommenacker, J. van de, J. Komdeur, and D. S. Richardson. 2011. Assessing the cost of helping: the roles of body condition and oxidative balance in the Seychelles warbler (Acrocephalus sechellensis).

Ergon, T., and B. Gardner. 2014. Separating mortality and emigration: modelling space use, dispersal and survival with robust-design spatial capture-recapture data. Methods in Ecology and Evolution 5:1327–1336.

Frenck, R. W., E. H. Blackburn, and K. M. Shannon. 1998. The rate of telomere sequence loss in human leukocytes varies with age. Proceedings of the National Academy of Sciences of the United States of America 95:5607–5610.

Griffiths, R., M. C. Double, K. Orr, and R. J. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–5.

Hammers, M., S. A. Kingma, K. Bebbington, J. Van de Crommenacker, L. G. Spurgin, D. S. Richardson, T. Burke, H. L. Dugdale, and J. Komdeur. 2015. Senescence in the wild: Insights from a long-term study on Seychelles warblers. Experimental Gerontology, doi: [10.1016/j.exger.2015.08.019](https://doi.org/10.1016/j.exger.2015.08.019).

Hammers, M., D. S. Richardson, T. Burke, and J. Komdeur. 2013. The impact of reproductive investment and early-life environmental conditions on senescence: support for the disposable soma hypothesis. Journal of Evolutionary Biology 26:1999–2007.

Haussmann, M. F., and N. M. Marchetto. 2010. Telomeres: Linking stress and survival, ecology and evolution. Current Zoology 56:714–727.

Haussmann, M. F., C. M. Vleck, and I. C. T. Nisbet. 2003. Calibrating the telomere clock in common terns, Sterna hirundo. Experimental Gerontology 38:787–789.

Heidinger, B. J., J. D. Blount, W. Boner, K. Griffiths, N. B. Metcalfe, and P. Monaghan. 2012. Telomere length in early life predicts lifespan. Proceedings of the National Academy of Sciences of the United States of America 109:1743–8.

Horn, T., B. C. Robertson, and N. J. Gemmell. 2010. The use of telomere length in ecology and evolutionary biology. Heredity 105:497–506.

Komdeur, J. 1991. Cooperative breeding in the Seychelles warbler. PhD Thesis, Cambridge University.

Komdeur, J. 1992. Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. Nature 358:493–495.

Komdeur, J. 1994. The effect of kinship on helping in the cooperative breeding Seychelles warbler (Acrocephalus sechellensis).

Komdeur, J., I. D. Bullock, and M. R. W. Rands. 1991. Conserving the Seychelles Warbler Acrocephalus sechellensis by translocation: a transfer from Cousin Island to Aride Island.

Komdeur, J., T. Piersma, K. Kraaijeveld, F. Kraaijeveld-Smit, and D. S. Richardson. 2004. Why Seychelles warblers fail to recolonize nearby islands: unwilling or unable to fly there? Ibis 146:298–302.

Mizutani, Y., N. Tomita, Y. Niizuma, and K. Yoda. 2013. Environmental perturbations influence telomere dynamics in long-lived birds in their natural habitat. Biology Letters 9:20130511.

Monaghan, P. 2014. Organismal stress, telomeres and life histories. Journal of Experimental Biology 217:57–66.

Monaghan, P., and M. F. Haussmann. 2006. Do telomere dynamics link lifestyle and lifespan? Trends in Ecology and Evolution 21:47–53.

Nettle, D., C. Andrews, and P. Monaghan. 2015a. Developmental and familial predictors of adult cognitive traits in the European starling. Animal Behaviour 107:239–248.

Nettle, D., P. Monaghan, R. Gillespie, B. Brilot, T. Bedford, and M. Bateson. 2015b. An experimental demonstration that early-life competitive disadvantage accelerates telomere loss. Proceedings of the Royal Society B: Biological Sciences 282:20141610. The Royal Society.

Nussey, D. H., D. M. Baird, E. L. B. Barrett, W. Boner, J. Fairlie, N. J. Gemmell, N. Hartmann, T. Horn, M. F. Haussmann, M. Olsson, C. Turbill, S. Verhulst, S. Zahn, and P. Monaghan. 2014. Measuring telomere length and telomere dynamics in evolutionary biology and ecology. Methods in Ecology and Evolution 5:299–310.

Pol, M. van de, and J. Wright. 2009. A simple method for distinguishing within-versus between-subject effects using mixed models. Animal Behaviour 77:753–758.

Price, L. H., H. T. Kao, D. E. Burgers, L. L. Carpenter, and A. R. Tyrka. 2013. Telomeres and early-life stress: An overview. Biological Psychiatry 73:15–23.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; R Foundation for Statistical Computing.

Reichert, S., F. Criscuolo, and S. Zahn. 2015. Immediate and delayed effects of growth conditions on ageing parameters in nestling zebra finches. The Journal of Experimental Biology 218:491–499.

Richardson, D. S., T. Burke, and J. Komdeur. 2003a. Sex-specific associative learning cues and inclusive fitness benefits in the Seychelles warbler. Journal of Evolutionary Biology 16:854–861.

Richardson, D. S., J. Komdeur, and T. Burke. 2003b. Avian behaviour: Altruism and infidelity among warblers. Nature 422:580.

Schultner, J., B. Moe, O. Chastel, C. Bech, and A. S. Kitaysky. 2014. Migration and stress during reproduction govern telomere dynamics in a seabird. Biology Letters 10:20130889.

Simons, M. J. 2015. Questioning causal involvement of telomeres in aging. Ageing Research Reviews, doi: [10.1016/j.arr.2015.08.002](https://doi.org/10.1016/j.arr.2015.08.002).

Spurgin, L. G., D. J. Wright, M. van der Velde, N. J. Collar, J. Komdeur, T. Burke, and D. S. Richardson. 2014. Museum DNA reveals the demographic history of the endangered Seychelles warbler. Evolutionary Applications 7:1134–1143.

Van de Crommenacker, J., J. Komdeur, T. Burke, and D. S. Richardson. 2011. Spatio-temporal variation in territory quality and oxidative status: A natural experiment in the Seychelles warbler (Acrocephalus sechellensis). Journal of Animal Ecology 80:668–680.

Von Zglinicki, T. 2002. Oxidative stress shortens telomeres. Trends in Biochemical Sciences 27:339–344.

Watson, H., M. Bolton, and P. Monaghan. 2015. Variation in early-life telomere dynamics in a long-lived bird: links to environmental conditions and survival. The Journal of Experimental Biology 218:668–674.

Wong, K. K., R. S. Maser, R. M. Bachoo, J. Menon, D. R. Carrasco, Y. Gu, F. W. Alt, and R. A. DePinho. 2003. Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. Nature 421:643–648.