**Strong cohort effects on early-life telomere length in a wild population**

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**Running head:** Early-life telomeres and survival

### Abstract

Understanding the short and long term costs of individual early-life experiences is fundamental to understanding life-history evolution. 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 early-life costs, and enhance our understanding of how poor conditions in early life are related to later-life survival. We tested how telomere dynamics are related to spatiotemporal variation in early-life conditions and later-life survival in the Seychelles warbler (*Acrocephalus sechellensis*), across multiple cohorts spanning 14 years. we found that, in accordance with other studies, telomere length and loss are greatest in early life. We then show that juvenile telomere lengths varies markedly among cohorts, with average telomere length varying by . We found no evidence that early-life social environment (number of helpers, group size) or ecological conditions (territory quality) were related to telomere length, although we found tentative evidence that telomere length varied among summer and winter breeding seasons. Finally, we found that increased survival later in life was associated with longer telomeres in early life, but this effect was at the cohort, rather than individual, level. Our results highlight the inmportance of cohort effects in studies of telomere length.

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

**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.

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### Introduction

Exposure to favourable environmental conditions during development and growth can confer fitness advantages later in life (so called 'silver spoon effects'; Grafen 1988; Monaghan 2008). Adult fitness can be affected by a range of early-life experiences, including the quality of the habitat and available resources (Madsen and Shine 2000; Van de Pol et al. 2006; Hayward et al. 2013), population density (Nussey et al. 2007; Douhard et al. 2013) and natural or anthropogenic environmental disturbance (Reid et al. 2003; Cartwright et al. 2014). Understanding the causes and consequences of these early-life experiences is key to understanding many ecological and evolutionary processes, including patterns of natural and sexual selection, population growth rates and even local extinction (Coulson et al. 2001; Roach and Carey 2014). Understanding silver spoon effects is therefore of central interest to ecologists, evolutionary biologists and conservationists.

While it is clear that silver spoon effects can occur, we have little understanding of how and under what conditions early-life environments will affect adult fitness. However , we do expect the later-life consequences of a good or poor start to vary among individuals, populations and species because the phenotypic consequences of an adverse environment can depend on an individual's initial condition, and genetic or epigenetic makeup (Hoffman and Hercus 2000; Richards 2006). Moreover, it is not always possible to fully quantify what constitutes a good or bad environment, and any 'hidden' environmental variation may obscure relationships between the early-life conditions that are measured and the resulting adult phenotypes. Indeed, the pervasiveness of silver spoon effects varies between species (Drummond et al. 2011), cohorts (Reid et al. 2003) and sexes (Wilkin and Sheldon 2009). We therefore need to understand, or at least be able to measure, how the environment affects individuals differentially within a population in order to elucidate the later-life consequences of early-life experience.

Telomeres - the protective caps on the ends of chromosomes - may provide a solution to this problem. 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 (Barrett and Richardson 2011; Simons 2015), there is now excellent 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 loss occurs in early life (e.g. Frenck et al. 1998; Haussmann et al. 2003), and that the extent of this telomere shortening is influenced by the conditions experienced during that period (Price et al. 2013; Monaghan 2014; Nettle et al. 2015b; Reichert et al. 2015). Importantly, 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 absolute 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 (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 link early-life environmental variation to late-life fitness in the Seychelles warbler. Because telomere dynamics are expected to reflect individual-level variation in the costs of early-life experiences, they may allow a more sensitive analysis of the effects of early-life environmental variation than would be possible with a direct comparison of how survival is affected by the early-life environment. With this in mind, we first conduct an exploratory analysis of how the environmental and social factors experienced in early life affect telomere dynamics. We then test the hypothesis that longer telomeres and lower rates of telomere shortening in early life are associated with greater survival.

### 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 has two breeding seasons, running from June-August (main breeding season) and December-February (minor breeding season), 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 territory quality estimates obtained across years are averaged to obtain a single value for each territory (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 thesholds 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 telomere length for all samples using a quantitative PCR (qPCR) assay of telomeres and a GPADH control gene, using the molecular methods outlined by Barrett *et al.* (2012), with one amendment. A change in batch of SYBR green forced us to raise the annealing temperature of the telomere reaction from 58oC to 61oC for the majority of samples. However, this did not affect final telomere length values (see below). We used the program LinRegPCR (Ruijter et al. 2009) to correct for baseline fluorescence and calculate efficiencies and Cq values for each sample replicate. Averaging of technical repeats was carried out using custom-made R scripts (available as supplementary material), excluding samples with Cq values differing by >0.5. We then calculated relative telomere length (RTL) for each sample using equation 1 in Pfaffi *et al* (**???**). We chose to use RTL rather than continuing with the previously used method for claculating absolute telomere length, as i) using RTL enabled us to run more samples per plate (as an oligo standard is not required), ii) the RTL method was less susceptible to batch effects (Appendix 1), and iii) very few other studies have calculated absolute telomere length, and our experience suggests that cross-species comparisons are unlikely to be reliable.

Inter-plate repeatability of final telomere lengths was assessed using the R package rptR (Schielzeth and Nakagawa 2011), and for all subsequent analyses we used mean telomere length per sample where we had repeats.

Telomere lengths were measured using a total of 1436 samples, . Of these, 1068 were taken cross-sectionally from birds caught within their first year of life, between 1998 and 2014. For a subset of first-year birds (n = 368 individuals) we had longitudinal data, with an additional sample taken as an adult. For these individuals we calculated the absolute amount of telomere loss between the first-year and adult samples by subtracting adult telomere length from early-life telomere length, as well as a rate of telomere loss by dividing this difference by the length of time (in days) between sampling events.

##### Statistical analyses

We performed all analyses using R version 3.0.1 (R Development Core Team 2011). Telomere length was log transfomred to fit assumptions of normality. We first explored how telomere length varied within the first year of life and over time using linear regression (telomere length vs age in months), and one-way ANOVA (telomere length vs cohort). With the longitudinal data, we tested how telomeres shorten with age by testing how telomere loss and rate of loss were related to the time interval between sampling events, using linear models. If telomeres are lost at a constant rate from early age, we expect the time between sampling events to be positively and linearly relaited to telomere loss no, and unrelated to rate of loss. If, however, telomeres are lost at a greater rate early in life we expect a non-linear or negative relationship between telomere loss and time between sampling events, and a significant (linear or non-linear) decrease in telomere rate of loss with time interval.

We then used a general linear mixed model approach to explore how spatial variation in early-life environmental and social conditions influenced telomere length within cohorts. Model averaging was carried out using the MuMIn package (version 1.10.5) in R (Bartoń 2012). We first created a full model containing the following explanatory variables: age (in months), tarsus length, sex, territory quality, season (summer or winter) and the number of helping and non-helping subordinate birds present in the natal territory. We also included interaction terms between age and all the other variables as telomere dynamics are epxected to vary within the first year of life (Heidinger et al. 2012). As random effects we included cohort, territory ID and qPCR plate ID. Model selection was then performed 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* = 368) we repeated the above analyses of telomere dynamics, replacing telomere length with telomere loss as the response variable, and excluding the plate ID random effect (as each measurement of telomere loss was based on two or more measurements, and so run on multiple plates).

We used Cox regression, implemented in the survival package in R (**???**), to test whether survival was related to individual-level telomere length and rate of loss. For the cross-sectional data we used lifespan (in years) as survival time, while for the longitudinal data we used remaining lifespan from the second sampling event. We ran these models with cohort as a frailty term to exclude cohort effects.

### Results

##### Early-life telomere dynamics

Efficiecies (mean ) for our telomere and GAPDH reactions were (1.79 0.04) and (1.92 0.06) respectively. Inter-plate repeatability of telomere length, based on XX samples measured at least twice, was 0.78 (CI = 0.73-0.82).

RTL dereased with age both within the first year of life (R2 = 0.03; F = 33.43; P < 0.001), and across the entire Seychelles warbler lifespan (R2 = 0.02; F = 48.32; P < 0.001; Fig. 1A). Longitudinal data showed that the rate of early-life telomere shortening also decreased with age (R2 = 0.02; F = 7.87; P = 0.005).

Both early-life RTL and rate of loss varied significantly among breeding seasons (one-way ANOVA, telomere length: F = 3.32; P < 0.001; Fig, 1C; telomere loss: F = 2.16; P < 0.001; Fig, 2). Variation in median RTL over breeding seasons in fledglings and subadults was not related temporal variation in territory quality (linear regression, R2 = 0.01; F = 0.30; P = 0.59), island-wide food availability (R2 = 0.02; F = 0.43; P = 0.52) or population density (R2 = 0.09; F = 2.31; P = 0.14).

##### Early-life environment and early-life telomere dynamics

The top model explaining variation in early-life RTL contained age, season (summer vs winter), tarsus length, and the interaction between age and tarsus length (Table S1). The top model was much better supported than a null model (AICc = 48.51), and all three effects were statistically significant (Fig. 2A). RTL was higher in winter compared to summer seasons (Fig. 2B). Tarsus length was negatively related to RTL in nestlings, unrelated to RTL in fledglings, and positively related to RTL in subadults (Fig. 2C).

Model averaging results from the longitudinal data are shown in Figure 3A. The top model explaining telomere loss contained age and season (Table S2). This model was a significantly better fit than the null model (AICc = 6.46). Juveniles born in winter seasons had higher rates of telomere shortening compared to birds born in summer seasons, but this effect was weak (Fig. 3B), although this effect was weak and confidence intervals overlapped zero (Fig. 3A). There was no evidence that tarsus length was related to telomere shortening in the same way that telomere length was (Fig. 3C).

##### Early-life telomere dynamics and survival

Telomere length in early life did not affect survival to adulthood (estimate = -0.24; P = 0.19), and there was no interaction effect between RTL and juvenile age on survival to adulthood (estimate = 0.00; P = 0.99). To separate out cohort-level RTL affected survival we calculated cohort-level RTL (i.e. the mean for each cohort) and added this term to a logistic regression. However, we found no effect of RTL on survival to adulthood (estimate = -0.42; P = 0.50), and no interaction with juvenile age (estimate = -0.10; P = 0.64)

Cox regression also showed no effect of individual-level or cohort-level RTL on survival later in life (individual-level: estimate = 0.05, P = 0.64; cohort-level: estimate = 0.63, P = 0.26). Using the longitudinal data, we found that the amount of telomere shortening experienced in early life had no effect on survival (estimate < 0.001, P = 0.806).

### Discussion

Here we use the long-term study of a closed population of Seychelles warblers to assess the relationships between early-life conditions, telomere length and survival. We find that while RTL varies

The clearest result from our study is that RTL varies among cohorts. Very few studies have shown that temporal variation in telomere dynamics occurs in natural populations, and to our knowledge the studies that have done so were limited to just two 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 does occur at the population level over longer time periods.

We suspect that the novelty in our finding temporal, environmentally-induced variation in telomere dynamics within a population is more due to a lack of available long-term datasets with telomere screening, rather than the Seychelles warbler being unique. Indeed, the environment on Cousin is benign in comparison with many regions outside the tropics, where populations undergo large fluctuations in size (e.g. Coulson et al. 2001). If our findings are replicated in other systems and population-level variation in early-life telomere dynamics is common in nature, this has potential conseaquences 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.

In the Seychelles warbler we found only weak evidence that any of the social or environmental vairables we measured affect juvenile RTL. This is surprising 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. Possible explanations for this finding include i) temporal variation in RTL in our data is so strong that we are unable to detect spatial trends, ii) within-cohort variation in RTL is explained by a variable that we have not included in our analyses, or iii) that effects are generally weak and levels of noise in our telomere measurments and/or ecological data preclude detection of significant effects. Future research should therefore examine, in a quantitative genetic framework, how genetic and environmental components, and their interactions, affect telomere dynamics and senescence in natural populations (Asghar et al. 2014; Becker et al. 2015).

Our data suggest that, in addition to age class, tarsus length and season had weak effects on juvenile RTL (Figs 3, 4). The tarsus effect most likely reflects the fact that in passerine birds tarsus length is correlated with age during the nestling stage (Ricklefs 1976). Telomere loss is most rapid early in life due to ongoing cell replication (Frenck et al. 1998), and a negative correlation between RTL and body size in early life is therefore expected. Indeed, that age class affects both tarsus length and RTL can be clearly seen in our data (Fig. 4B). The difference in RTL between birds born in summer and winter seasons is more surprising, especially given the directionality of the effect. We find that birds born in winter seasons having longer telomeres (Fig. 4A), while the opp. One possible explanation for this is that parents in good condition are more likely to breed

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.

Finally, our study highlights some of the diffculties asosciated with studying telomere dynamics in natural populations.

Our longitudinal dataset was limited, both in terms of sample size and resolution (i.e. time between sampling events). Seychelles warblers are rarely sampled more than once within their first year of life, so much of the telomere shortening that occurs in early life will be missed with our sampling regime. It is likely, therefore that only very strong effects of environmental variation on telomere loss will be detected in this dataset. Thus telomere length constitutes a better indicator of early-life stress.

There are numerous avenues for future research into telomere evolutionary ecology in this system and others. 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.

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### 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.

Cartwright, S. J., M. A. C. Nicoll, C. G. Jones, V. Tatayah, and K. Norris. 2014. Anthropogenic natal environmental effects on life histories in a wild bird population. Current Biology 24:536–40.

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.

Coulson, T., E. A. Catchpole, S. D. Albon, B. J. Morgan, J. M. Pemberton, T. H. Clutton-Brock, M. J. Crawley, and B. T. Grenfell. 2001. Age, sex, density, winter weather, and population crashes in Soay sheep. Science 292:1528–1531.

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).

Douhard, M., J.-M. Gaillard, D. Delorme, G. Capron, P. Duncan, F. Klein, and C. Bonenfant. 2013. Variation in adult body mass of roe deer: early environmental conditions influence early and late body growth of females. Ecology 94:1805–1814.

Drummond, H., C. Rodríguez, and D. Oro. 2011. Natural ’poor start’ does not increase mortality over the lifetime. Proceedings of the Royal Society B: Biological Sciences 278:3421–3427.

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.

Grafen, A. 1988. On the uses of data on lifetime reproductive success. *in* T. Clutton-Brock, ed. Reproductive success. University of Chicago Press, Chicago.

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](http://dx.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.

Hayward, A. D., I. J. Rickard, and V. Lummaa. 2013. Influence of early-life nutrition on mortality and reproductive success during a subsequent famine in a preindustrial population. Proceedings of the National Academy of Sciences of the United States of America 110:13886–91.

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.

Hoffman, A. A., and M. J. Hercus. 2000. Environmental Stress as an Evolutionary Force. BioScience 50:217–226. Oxford University Press.

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.

Madsen, T., and R. Shine. 2000. Silver spoons and snake body sizes: prey availability early in life influences long‐term growth rates of free‐ranging pythons. Journal of Animal Ecology 69:952–958.

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. 2008. Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society B: Biological sciences 363:1365.

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., L. Kruuk, A. Morris, and T. Clutton-Brock. 2007. Environmental conditions in early life influence ageing rates in a wild population of red deer. Current Biology 17:R1000–R1001.

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.

Reid, J. M., E. M. Bignal, S. Bignal, D. I. McCracken, and P. Monaghan. 2003. Environmental variability, life-history covariation and cohort effects in the red-billed chough Pyrrhocorax pyrrhocorax. Journal of Animal Ecology 72:36–46.

Richards, E. J. 2006. Inherited epigenetic variation–revisiting soft inheritance. Nature Reviews Genetics 7:395–401.

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.

Ricklefs, R. E. 1976. Growth rates of birds in the humid new world tropics. Ibis 118:179–207.

Roach, D. A., and J. R. Carey. 2014. Population biology of aging in the wild. Annual Review of Ecology, Evolution, and Systematics 45:421–443.

Ruijter, J. M., C. Ramakers, W. M. H. Hoogaars, Y. Karlen, O. Bakker, M. J. B. Van den hoff, and A. F. M. Moorman. 2009. Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Research 37.

Schielzeth, H., and S. Nakagawa. 2011. rptR: Repeatability for Gaussian and non-Gaussian data. R package version 0.6.404/r42.

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](http://dx.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.

Van de Pol, M., L. W. Bruinzeel, D. Heg, H. P. Van der Jeugd, and S. Verhulst. 2006. A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of oystercatchers (Haematopus ostralegus). Journal of Animal Ecology 75:616–626.

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.

Wilkin, T. A., and B. C. Sheldon. 2009. Sex differences in the persistence of natal environmental effects on life histories. Current Biology 19:1998–2002.

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.