Telomere heritability and parental age at conception effects in a wild avian population

Running title: Heritability of telomere length

Authors: Alexandra M. Sparks[1], Lewis G. Spurgin[2], Marco van der Velde[3], Eleanor A. Fairfield[2], Jan Komdeur[3], Terry Burke[4], David S. Richardson[2,5*], Hannah L. Dugdale[1,3*]

Affiliations:

[1] Faculty of Biological Sciences, School of Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom

[2] School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, United Kingdom

[3] Behavioural and Physiological Ecology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, 9747 AG, The Netherlands

[4] Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, United Kingdom

[5] Nature Seychelles, Victoria, Mahé, Republic of Seychelles

* H.L.D. and D.S.R. are joint senior author

Corresponding author: Alexandra M. Sparks, A.M.Sparks@leeds.ac.uk

Abstract:

Individual variation in telomere length is predictive of health and mortality risk across a range of species. However, the relative influence of environmental and genetic variation on individual telomere length in wild populations remains poorly understood. In previous studies, heritability of telomere length has primarily been calculated using parent-offspring regression, but shared environments can confound such estimates. Furthermore, associations with age and parental age at conception effects are typically not accounted for but can also bias heritability estimates. To control for these confounding variables, quantitative genetic 'animal models' can be used. However, the few studies on wild populations using this approach have been restricted by power. Here, we investigated the heritability of telomere length and parental age at conception effects in the Seychelles warbler using 2664 telomere length measures from 1318 birds over 20 years and a multi-generational pedigree. We found a weak negative within-paternal age at conception effect (as fathers aged, their offspring had shorter telomeres) and a weak positive between-maternal age at conception effect (females that survived to older ages had offspring with longer telomeres). While parent–offspring regressions did not detect heritability, animal models provided evidence that heritability of telomere length was low in this population. Environmental and technical variation largely influenced telomere length and would have biased heritability estimates if unaccounted for. Estimating the heritability of telomere length is complex, requiring large sample sizes and accounting for confounding effects in order to improve our understanding of the evolutionary potential of telomere length in the wild.

Keywords: telomere length, heritability, animal model, paternal age at conception, maternal age at conception, Seychelles warbler

Introduction

A complete understanding of the relative impact of genetic and environmental effects on senescence rates requires quantifying individual variation in senescence rates, but this is difficult to achieve, especially in wild populations (van de Pol and Verhulst 2006; Nussey et al. 2008; Charmantier et al. 2014). However, the identification of biomarkers, such as telomeres that reflect an individual's intrinsic state and mortality risk (Wilbourn et al. 2018), have facilitated this (Nakagawa et al. 2004). Telomeres, short repetitive DNA elements that protect the ends of eukaryotic linear chromosomes (Blackburn 1991), shorten with each cell cycle due to the end replication problem (Levy et al. 1992) and other mechanisms including oxidative damage (von Zglinicki 2002). Critically short telomeres can trigger cellular senescence (Harley et al. 1992; Campisi 2005) which may lead to organismal senescence (López-Otín et al. 2013). However, telomeres can also be extended by telomerase (Greider and Blackburn 1989) and alternative lengthening (Cesare and Reddel 2010). Telomere shortening occurs with age in a wide range of species (e.g. Salomons et al. 2009; Aubert et al. 2012). Furthermore, whether causal, or just correlational (Simons 2015; Young 2018), telomere length relative to age positively predicts health (Boonekamp et al. 2013; Blackburn et al. 2015) and survival/lifespan within species (Barrett et al. 2013; Wilbourn et al. 2018). Consequently, telomeres are increasingly used in evolutionary ecology studies as a biomarker of senescence and to measure an individual's physiological response to their environmental experiences (Bize et al. 2009; Bauch et al. 2012; Bebbington et al. 2016; Fairlie et al. 2016).

A better understanding of the drivers of individual variation in telomere length is important if we are to use them as a biomarker of health and senescence within populations (Dugdale and Richardson 2018). Initial telomere length is inherited from each parent (Delgado et al. 2019). As

68 a mother's gametes are produced prenatally, whereas fathers produce sperm throughout their life,
69 paternal age at conception may impact the telomere lengths of their offspring (Eisenberg and
70 Kuzawa 2018). There is cross-sectional evidence from humans that sperm telomere length is
71 positively correlated with age and older fathers have offspring with longer telomeres (Unryn et
72 al. 2005; Kimura et al. 2008; Aston et al. 2012; Eisenberg et al. 2012; Broer et al. 2013). Such
73 effects may be due to the activity of telomerase in the testes resulting in elongated telomeres with
74 age (Kimura et al. 2008; Aviv and Susser 2013). An alternative (not mutually exclusive)
75 hypothesis is the selective survival or proliferation of germ stem cells with longer telomeres
76 (Kimura et al. 2008; Hjelmborg et al. 2015). However, studies in non-human vertebrates,
77 including a longitudinal study in jackdaws (Bauch et al. 2019), report conflicting results; while
78 some have also found a positive correlation between offspring telomere length and paternal
79 (Eisenberg et al. 2017) or maternal age (Asghar et al. 2015), others have found negative paternal
80 age correlations (Olsson et al. 2011; de Frutos et al. 2016; Criscuolo et al. 2017; Bouwhuis et al.
81 2018; Noguera et al. 2018; Bauch et al. 2019) or no parental age effects (Heidinger et al. 2016;
82 Froy et al. 2017; McLennan et al. 2018; Belmaker et al. 2019; van Lieshout et al. 2020*a*). Work
83 on a wider range of species, using longitudinal data, is required to identify the drivers of
84 variation in parental age at conception effects.

85

86 In addition to parental age effects, genetic variation also influences the maintenance of telomeres
87 from the first mitotic division, and thus telomere dynamics across an individual's lifetime
88 (Delgado et al. 2019; Eisenberg 2019). Environmental effects and life-history events are also
89 associated with telomere shortening due to the stress they exert on the organism, and such effects
90 will accumulate with age (Hall et al. 2004; Heidinger et al. 2012). The majority of studies
91 looking to quantify the contribution of genetic variation to telomere length are in human
92 populations which typically implicate significant heritability (Dugdale and Richardson 2018). It

93 is, however, difficult to interpret heritability estimates from human studies where processes, such
94 as industrialisation and medical interventions, limit their evolutionary interpretation, and in
95 captive or laboratory populations that exist in controlled environments. Importantly, biologists
96 wanting to understand the ecological and evolutionary significance of telomere variation will be
97 interested in studying telomere heritability in wild populations, which are experiencing natural
98 environmental variation and where natural selection is occurring. To date, very few studies have
99 attempted to separate genetic from environmental contributions to variation in telomere length
100 within wild populations, and most have been restricted in terms of small sample sizes.
101 Consequently, our understanding of the heritability of telomeres in wild populations is limited
102 (Dugdale and Richardson 2018). This is important as the amount of additive genetic variance
103 underlying a trait, such as telomere length, limits the variation that selection can act on and,
104 therefore, a trait's evolutionary potential (Lynch and Walsh 1998).

105

106 In wild populations, telomere length heritability estimates range from 0 to 1 (Dugdale and
107 Richardson 2018). Heritability estimates have, however, been based primarily on parent–
108 offspring regressions, which assume that the similarity between parents and offspring is genetic,
109 when, in fact, relatives often also share environments. These shared environmental effects,
110 including cohort and maternal effects (Asghar et al. 2015; Becker et al. 2015), will artificially
111 inflate heritability estimates (Kruuk and Hadfield 2007; Kruuk et al. 2008). Additionally,
112 telomere length will change throughout life, so measures across the lifetimes of individuals will
113 be the product of inherited telomere length, attrition, and restoration/lengthening (Dugdale and
114 Richardson 2018). Few telomere studies have taken individual age at sampling into account
115 (Reichert et al. 2015), or sampled both offspring and parents at the same age, as both sampling
116 and accurate ageing are difficult in wild populations (but see Becker et al, 2015 and Asghar et al.

2015). Furthermore, parental age at conception may also impact the telomere length of their offspring (Eisenberg et al. 2012). Subsequently, it is unclear whether the variation in heritability estimates of telomere length in wild populations reflects true variation, or methodological or analytical differences between studies.

Quantitative genetic "animal models" offer a strong analytical framework to estimate the relative effects of additive genetic and environmental variation on phenotypic traits (Kruuk 2004). Animal models utilise the relationships in a pedigree to estimate additive genetic variance, thus maximising data and increasing the power to detect heritabilities (Wilson et al. 2010). Additionally, animal models can account for, and estimate the contribution of, other factors known to influence telomeres, to get more accurate estimates of the proportion of phenotypic variance due to additive genetic effects. However, animal models require considerable sample sizes (Wilson et al. 2010). Of the three wild vertebrate studies estimating telomere length heritability using a pedigree-based animal model, two attempted to partition shared maternal environment effects but one did not converge and, therefore, could not separate these effects (Asghar et al. 2015) and the other explained litter variation so was not included (Becker et al. 2015). There is a clear need for studies assessing the heritability of telomere length using large datasets with multigenerational pedigrees and animal model approaches (Dugdale and Richardson 2018).

In this study, we used the long-term individual-based multi-generational data from an isolated population of the cooperatively breeding Seychelles warbler (*Acrocephalus sechellensis*) to investigate additive genetic and environmental variance components underlying telomere length. Telomere length declines with age and adult survival is positively associated with telomere

length, independent of age, in this population (Barrett et al. 2013). Telomere loss is greatest in early life in the Seychelles warbler and shows strong cohort effects, and telomere length is positively associated with food abundance (Spurgin et al. 2018). However, telomeres also appear to elongate within individuals in this population (Spurgin et al. 2018). Telomere dynamics have helped reveal the costs of factors such as inbreeding (Bebbington et al. 2016) and social conflict (Bebbington et al. 2017). In the Seychelles warbler, telomere length is therefore an important biomarker of condition and senescence and is impacted by environmental conditions.

Here, we estimate the heritability of telomere length in the Seychelles warbler using 2664 telomere measures from 1318 birds within a 10-generation genetic pedigree. First, we test for parental age at conception effects on offspring telomere length accounting for the age at which offspring were sampled. Next, we estimate the heritability of telomere length using parent–offspring regressions, and investigate how heritability estimates differ between maternal–, paternal– and mid-parent–offspring analyses, when measurements were taken at different ages. We predict that heritability estimates will be higher when sampled at younger ages, since these samples will be closer to the telomere length initially inherited from parents. We then compare heritability estimates from the regressions to those gained from animal models where we control for expected confounding effects. We predict estimates of heritability to be higher in parent–offspring regressions compared to animal models, and higher when we included fewer common environmental effects (due to the upward biasing of heritability as a result of shared environments). Finally, we discuss the broader implications of our results for our understanding of the evolutionary potential of telomeres in this population.

Materials and Methods

Study system

The Seychelles warbler is a small passerine endemic to the Seychelles archipelago (Komdeur et al. 1991). The entire population (ca. 320 adult individuals in 115 territories) on Cousin island (29 ha; 04′20′S, 55′40′E) has been monitored extensively since 1985 (Komdeur 1992; Richardson et al. 2007; Hammers et al. 2019; Raj Pant et al. 2019). Seychelles warblers defend year-round territories in which a dominant male and female reside and most clutches contain 1 egg (Komdeur 1994; Richardson et al. 2001). The main breeding season runs from June to September, although some pairs also breed between January and March (Komdeur et al. 1991; Komdeur and Daan 2005). Senescence has been documented in the Seychelles warbler (Hammers et al. 2015) with age-dependent declines in both reproduction and survival (Hammers et al. 2012, 2013). Seychelles warblers are largely insectivorous, and variation in rainfall drives variation in insect abundance (Komdeur and Daan 2005) which was positively associated with telomere length (Spurgin et al. 2018). In addition, the study can compare genetic and social parent effects on telomere variation, due to the presence of extra-group paternity (41% of offspring) and subordinate female cobreeding (11% of offspring) (Raj Pant et al. 2019).

All protocols were ethically reviewed and approved by the BIO Ethical Review Committee, University of East Anglia, UK, and ratified by the University of Leeds. Each breeding season as many birds as possible are caught using mist nets and all territories monitored for the presence and identity of individually colour-ringed birds. The majority (96%) of individuals have been individually marked with a British Trust for Ornithology ring and unique colour ring combinations (Richardson et al. 2001). Age of unringed birds was estimated using eye colour (Komdeur et al. 1991), and where available lay, hatch or fledge dates. Since 1995, blood samples

(ca. 25 ul) have been taken and stored at room temperature in absolute ethanol, thus allowing molecular sexing, parentage assignment (Richardson et al. 2001; Hadfield et al. 2006), pedigree construction (Edwards et al. 2017) and telomere length measurement (Barrett et al. 2013). The population is virtually closed (<0.1% dispersal; (Komdeur et al. 2004)) and extrinsic mortality is low, so birds live long lives (maximum observed lifespan = 18 years). Further, the population is intensively monitored with high annual resighting rates (ca. 0.92±0.02 for birds ≤ 2 years and 0.98±0.01 for older birds, Brouwer et al. 2010), so accurate birth and death years are known (Hammers et al. 2015).

Telomere data

We used the telomere dataset generated in Spurgin et al. 2018, which included birds caught and blood sampled between 1995 and 2014, when the data were most complete. Relative telomere length (RTL) was estimated using qPCR (Barrett et al. 2013; Bebbington et al. 2016; Spurgin et al. 2018). Our cleaned dataset included 2664 samples from 1318 individuals that passed quality control (Bebbington et al. 2016) and filtering steps (telomere cq ≥25 and cq replicate difference ≥0.5; GADPH cq ≤21 but ≥ 26 and cq replicate difference ≥0.5; RTL values ≥3). There were no storage time effects on telomere length (Spurgin et al. 2018). To investigate plate variance (by including qPCR plate as a random effect in our statistical models), where samples had replicates across plates (n=388), an RTL value for a given blood sample was taken at random.

Genetic pedigree

Protocols for genotyping, quality control tests and parentage assignments (*MasterBayes* 2.5.2; (Hadfield et al. 2006)), and pedigree statistics are provided in the supplementary information (Supplementary parentage methods, Figures S1–3 and Tables S1–3). Parentage was assigned at *p*

≥ 0.8. The pruned pedigree, calculated using *Pedantics* 1.7 (Morrissey and Wilson 2010), included parentage assignments for individuals born 1992–2014 and contained 1482 informative individuals for telomere length with 1217 maternities and 1268 paternities (Table S3).

Statistical analyses

Paternal age at conception effects on offspring telomere length

Statistical analyses were performed in R 3.5.3 (R Core Team 2019). We first investigated associations between parental ages at conception and RTL in offspring using linear mixed effects models with Gaussian error distribution in *lme4* 1.1-21 (Bates et al. 2015). RTL was square root transformed to improve linear mixed model fits, and in each model subset RTL was subsequently z-transformed for comparability of telomere studies (Verhulst 2020). Collinearity between the fixed effects was checked by calculating Variance Inflation Factors (VIF); all VIFs were <3. We fitted offspring RTL across all ages that offspring were sampled at as the response variable and included offspring sex (factor), offspring age in years (log-transformed for all ages and juvenile model following, Spurgin et al. 2018), parental age at conception (maternal and paternal) and technician identity (factor: 2 levels) as our fixed effects. Random effects included offspring identity, maternal identity, paternal identity, capture season ID and qPCR plate.

Based on our dataset and model structure we had ≥80% statistical power to detect paternal age at conception effect sizes of ≥0.02 (Figure S4) using a simulation-based power analysis in the package *simr* 1.0.5 (Green and MacLeod 2016). This was equivalent to a correlation coefficient of 0.059 (following Froy et al. 2017) which is sufficient power to detect paternal age at

conception effects of the correlation coefficients previously published (De Meyer et al. 2007; Nordfjäll et al. 2010; Eisenberg et al. 2012, 2017). There was considerable variation in maternal and paternal ages at conception (Figure S5A–B) and a significant but weak correlation between the two ($r=0.12$, $t_{1154}=4.08$, $p<0.001$, Figure S5C) which allowed us to include both variables in the same model. Significance was determined using likelihood ratio tests where the fixed effect of interest was dropped from the full model.

We also investigated paternal (PAC) and maternal age at conception (MAC) effects where offspring RTL was restricted to the first measurement as a nestling (n=304), or all juvenile measures (<1 year old, n=1137 measures of 958 offspring). The model structure was identical to the model of all ages, except offspring identity was not included as a random effect for the chick model, and for the juvenile model paternal identity was not included to allow model convergence.

To investigate whether effects on offspring telomere length were driven by within-parental age rather than between-parental age (selective disappearance) at conception effects, we used within-subject centering (van de Pol and Wright 2009). To the model of RTL across all ages, we first removed PAC and MAC and included mean age at conception per parent (between-parental age effects) and the deviation from the mean age at conception of the parent (within-parental age effects). To test whether the within and between slopes differed from each other, we included parental age at conception (within-individual age effects) and mean parental age at conception (difference between the within and between-individual slopes) in a second model. The significance of mean parental age at conception in this second model indicates that these within and between slopes in the first model are significantly different (van de Pol and Wright 2009).

Heritability of telomere length

We first investigated heritability of telomere length with parent–offspring regressions using a general linear model where offspring RTL was the response variable and parent RTL was a covariate. We used a frequentist approach since no random effects were included. Using *pwr* 1.2-2 (Champely 2018) we had ≥80% power to detect correlations ≥0.195 and ≥0.104, using the minimum (n=165) and maximum sample sizes (n=585) respectively, at a significance threshold of 0.05 (for all sample sizes see Table S4). We used mother–offspring, father–offspring and mid-parent–offspring regressions of RTL to explore how these affected our heritability estimates as well as investigating maternal/paternal transmission differences or the presence of potential maternal/paternal effects. We also investigated how these heritability estimates changed when we used telomere measures taken at all ages or just juvenile ages (<1 year) for both parents and offspring. For each analysis, a mean RTL measure was taken for each offspring and parent either using RTL across all ages or using only RTL measures taken of the individual when they were a juvenile. To avoid pseudoreplication due to the presence of multiple offspring from the same parent, mean offspring or mid-offspring telomere length was used for each mother, father or parent pair. Hence, a mean of the mean RTL from their offspring was taken. Heritabilities were calculated as twice the slope of maternal or paternal RTL on offspring RTL in mother–offspring or father–offspring regressions, or equal to the slope of midparent RTL on offspring RTL in the mid-parent–offspring regressions (Lynch and Walsh 1998).

Finally, we investigated heritability of telomere length in quantitative genetic "animal models" in *MCMCglmm* 2.26 (Hadfield 2010). We used a Bayesian approach to provide accurate estimates of our variance components. Our pruned pedigree had ≥80% power to detect heritabilities of

283 ≥0.17 (Figure S6), determined in *Pedantics* 1.7 (Morrissey and Wilson 2010). These univariate
284 models were fitted with RTL (non-transformed) as the response variable and had an increasingly
285 complex fixed and random effect structure. This allowed us to test for confounds between
286 random effects, and to investigate how the inclusion of random effects affected our estimates of
287 heritability. Model 1 included only individual identity to account for repeated measures (to
288 calculate between-individual variation or 'repeatability'). In model 2, individual identity was
289 partitioned into additive genetic and permanent environment components using the pruned
290 pedigree. In model 3, we included fixed effects of sex (factor), age (log-transformed following
291 Spurgin et al. 2018) and technician (factor: 2 levels) to investigate how heritability was impacted
292 by the inclusion of fixed effects (following: Wilson 2008; de Villemereuil et al. 2018). In model
293 4 we estimated technical variance by adding qPCR plate ID as a random effect. We subsequently
294 added maternal (model 5) and paternal (model 6) identity, determined from the genetic pedigree,
295 to investigate parental effects underlying telomere length. Maternal effects have previously been
296 observed in other species (Asghar et al. 2015), and maternal inbreeding effects, but not paternal
297 inbreeding effects, on offspring telomere length have been documented in our population
298 (Bebbington et al. 2016). We then added the random effects of season of capture (model 7) and
299 current territory (model 8), to account for spatio-temporal factors associated with telomere length
300 (Spurgin et al. 2018). Finally, we tested for early-life effects of birth season (model 9) to account
301 for long-lasting effects of natal conditions on telomere variation. Although we had information
302 on natal territory, models including natal territory did not converge, and simpler models
303 suggested that natal territory explained no variance in RTL. We used default priors for fixed
304 effects, while for the random effects (except for the residual variance structure which were
305 inverse-Wishart priors, where V=1, n=0.002) we applied parameter expanded priors (with V=1,
306 nu=1, alpha.mu=0 and alpha.V=1000) as the variance estimates were close to zero (Hadfield
307 2019). We ran our models with a variety of iterations (Models 1-3: 1.2×10^6 iterations, burn-

in=2×10^5, thinning=500; Models 4-5: 2.4×10^6 iterations, burn-in=4×10^5, thinning=1000; Models 6-9: 3.6×10^6 iterations, burn-in=6×10^5, thinning=1500). To assess convergence of *MCMCglmm* models, we checked: autocorrelation $r<0.1$, effective sample sizes >1000, Heidelberger and Welch's tests were passed and Geweke tests were passed. For estimates of the fixed effects and random effects we took the posterior mode of the posterior distributions. We defined fixed effects as significant if the 95% credible intervals of the posterior modes did not overlap zero. Heritability estimates, and the proportion of phenotypic variance explained by other variance components, were calculated by taking the posterior mode of the ratio of the additive genetic variance to total phenotypic variance for each sample of the posterior distribution.

To confirm the robustness of our estimates of telomere length heritability, we also ran the final model in a frequentist framework using ASReml-R 3 (Butler et al. 2009) using the same structure as model 9. Significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test using twice the absolute difference in log-likelihoods between the two models.

We then tested whether parental effects were present when using the social (i.e. the dominant breeding pair) rather than the genetic parents, which is possible due to extra-group paternity (41% of offspring) and co-breeding (11% of offspring) (Raj Pant et al. 2019). To do this, we compared the model with genetic parents (model 7) to that where the genetic parents were replaced with social parents (model 10: specifications: 1×10^7 iterations, burn-in=4×10^6, thinning=3000) using model 7's structure, since other random effects in models 8 and 9 explained a small proportion of the phenotypic variance (see Results).

Results

Maternal and paternal age at conception were not significantly associated with offspring relative telomere length when using telomere lengths across all ages, or when the dataset was restricted to the first offspring measurements taken as chicks, or when all measurements were taken from juvenile offspring (<1 year old; Figure S7, Table S5). However, when parental age at conception effects were separated into within- versus between-parental age effects for lifelong RTL, there was a significant and negative within-paternal age effect and a significant and positive between-maternal age effect (Table 1, Figure 1). As fathers aged the offspring they produced had progressively shorter telomeres, while females that survived to older ages had offspring with longer telomeres (Figure 1). Within- versus between-parental age slopes were significantly different from each other for both maternal and paternal age at conception (Table 1). However, both the within-paternal and between-maternal age effects on offspring RTL were small (Figure 1). There was no difference in lifelong RTL between sexes, but there was a logarithmic association with age and an effect of technician (Table S5).

Using parent–offspring regression techniques, we found no evidence for mother–offspring, father–offspring or mid-parent–offspring resemblance and hence no heritability of RTL using mean telomere measures across all ages. Further, there was no evidence for parent–offspring resemblance when using just mean juvenile (<1 year old) telomere measures of both offspring and parents (Figure 2, Table S4).

We estimated heritability with a quantitative genetic animal model using a hierarchical approach (Figure 3). Within-individual repeatability of RTL, the amount of variance due to individual identity, was low across all models and ranged from 0.056 (95% CrI: 0.016-0.092; Table 2: Model 9) to 0.136 (95% CrI: 0.078-0.195; Table S6: Model 1). As repeatability sets the upper limit on standard heritability (when indirect genetic effects are not considered), heritability estimates were also low across all models. RTL heritability was 0.080 (95% CrIs: 0.041-0.144; Table S6: Model 2) in the simplest model and was estimated as 0.031 (95% CrIs: <0.001-0.067) after the inclusion of all fixed and random effects in the final model (Figure 3, Table 2: Model 9). We found a small effect of season of capture and moderate qPCR plate effects in the final model (Table 2). There was no evidence for maternal or paternal effects, territory effects or birth season effects (Table 2). If plate variance was not included in the total phenotypic variance, since it represents technical but not biological variance (following de Villemereuil et al. 2018), individual repeatability was 0.077 (95% CrI: 0.028-0.125), heritability was 0.048 (95% CrI: <0.001-0.087), and capture season was 0.036 (95% CrI: 0.018-0.101) in the final model. A frequentist approach using ASReml-R produced similar results: repeatability was 0.057 ± 0.023 SE and heritability was low but significant at 0.041 ± 0.018 SE (Table S7). Without plate included, repeatability was 0.074 ± 0.030 SE and heritability was 0.053±0.023 SE.

Parental effects were compared when social parents (dominant breeding pair) or genetic parents (from the pedigree) were included. Maternal and paternal effects were close to zero in both models and did not differ significantly between models based on overlapping 95% credible intervals (Table 3). Heritability estimates were not significantly different based on the 95% credible intervals of the two models (Table 3).

Discussion

We found a negative but weak within-paternal age at conception effect and a positive but weak between-maternal age at conception effect on offspring telomere length in the Seychelles warbler, which adds to the growing literature reporting mixed results in wild populations (Asghar et al. 2015; Belmaker et al. 2019; Eisenberg 2019). Simple mother–offspring, father–offspring or mid-parent–offspring regressions did not provide evidence for telomere heritability in this population. However, animal models indicated a low heritability of telomere length, small catch season effects and moderate experimental effects in the form of qPCR plate effects.

A number of human studies have documented a positive cross-sectional association between paternal age at conception and offspring telomere length (Unryn et al. 2005; Eisenberg et al. 2012; Broer et al. 2013). Including just paternal and maternal age at conception in the model, we found no evidence for cross-sectional parental age at conception effects on offspring telomere length in the Seychelles warbler, even with sufficient power to detect paternal age at conception effects of the correlation coefficients previously published (De Meyer et al. 2007; Nordfjäll et al. 2010; Eisenberg et al. 2012, 2017). However, using within-subject centering we found weak but significant within-paternal age at conception and between-maternal age at conception effects on offspring telomere length. In contrast to studies in humans (Unryn et al. 2005; Eisenberg et al.

2012; Broer et al. 2013), we found that males produced offspring with shorter telomere lengths as they aged, and females that lived longer tended to have offspring with longer telomere lengths. However, both these effects were relatively small, and explained a very small amount of variation in offspring telomere length.

Despite the consistency in human studies, studies in non-human vertebrate populations are providing mixed evidence of paternal age at conception effects (Eisenberg 2019). While a few have documented positive paternal age at conception effects (Eisenberg et al. 2017; Dupont et al. 2018), most find a negative paternal age at conception effect (Olsson et al. 2011; de Frutos et al. 2016; Criscuolo et al. 2017; Bouwhuis et al. 2018; Noguera et al. 2018; Bauch et al. 2019). Furthermore, many studies have documented no parental age at conception effects (Heidinger et al. 2016; Froy et al. 2017; McLennan et al. 2018; Belmaker et al. 2019; van Lieshout et al. 2020*a*), while one study found a positive maternal but no paternal age at conception effect (Asghar et al. 2015). In studies investigating telomere heritability, only one has controlled for parental age at conception effects (Asghar et al. 2015). In our study we did not control for parental age at conception effects in our animal model, since overall effects were not significant and within and between parental age effects were small. However, where parental age at conception effects are significant and large in a population, these effects should be controlled for to obtain accurate estimates of the heritability of telomere length (Dugdale and Richardson 2018).

The majority of studies investigating the heritability of telomere length in wild populations have relied on parent–offspring regression techniques, and have found both significant and non-significant heritabilities ranging from 0 to 1 (Dugdale and Richardson 2018). While these could

reflect true differences in heritability estimates in different populations they may also be driven by methodological issues. For instance, despite clear relationships between telomere length and age, many studies have measured parents or offspring telomere lengths at different ages, or have controlled for age of sampling in different ways (Dugdale and Richardson 2018). In a king penguin *Aptenodytes patagonicus* study, chicks were measured at 10, 70, 200 and 300 days old, while mothers were sampled during the breeding season in which chicks were hatched. The authors found a positive association between maternal and offspring telomere length, but only when chicks were 10 days old, indicating that age of measurement can impact heritability estimates (Reichert et al. 2015). Therefore, we investigated how our estimates of telomere heritability differed when using parent–offspring regressions with RTL measured across all ages or just as juveniles. We predicted that resemblance would be higher when both parents and offspring were measured as juveniles, since these would be closest to initial telomere length and the accumulation of environmental effects would be lowest (Dugdale and Richardson 2018). However, we found no evidence of heritability of telomeres using either lifelong telomeres measures or just juvenile telomere measures. Our results contrast with studies which typically show significant mother–offspring rather than father–offspring regressions, indicative of heritable and/or maternal effects (either environmental or genetic in origin) underlying telomere variation (Asghar et al. 2015; Becker et al. 2015; Reichert et al. 2015). Our parent–offspring results indicated a very low or non-significant heritability, and/or a lack of parental effects underlying telomere variation in the Seychelles warbler.

We subsequently investigated heritability using an animal model approach. Telomere length had a low between-individual repeatability (without plate variance: 0.077; 95% CrI: 0.028-0.125) and a low heritability (without plate variance: 0.048; 95% CrI: <0.001-0.087). The three studies in wild populations that have previously estimated heritability using animal models found either no

444 significant heritable variation underlying telomere length variation (Becker et al. 2015), very low
445 heritability estimates (0.011, 95% CrIs: <0.001-0.042, to 0.060, 95% CrIs: 0.023-0.106
446 depending on prior specification, Foley et al. 2020) or a large heritability of 0.48 (95% CIs: 0.24-
447 0.72, Asghar et al. 2015). However, power analyses were not provided, and sample sizes were
448 generally relatively small for animal models (N≤504; except see Foley et al. 2020). Large
449 samples sizes are particularly needed in order to fully separate additive genetic effects from
450 common environment effects such as maternal effects (Becker et al. 2015). High within-
451 individual variation in telomere measures has been previously documented in other longitudinal
452 studies (Fairlie et al. 2016; Foley et al. 2020) including in the Seychelles warbler (Spurgin et al.
453 2018). Our estimate of repeatability (between-individual variation) was low and sets the upper
454 limit on ordinary narrow-sense heritability (Bijma 2011).

455

456 The low heritability of telomere length in the Seychelles warbler is consistent with individuals
457 with longer telomeres having a higher probability of surviving until the next year, independent of
458 age (Barrett et al. 2013). It is possible that selection for longer telomeres in this population has
459 reduced the genetic variation, and hence the heritability of this trait (Falconer and Mackay 1996).
460 Further, the large contribution of environmental effects to telomere length is supported by the
461 positive association between food availability and telomere length (Spurgin et al. 2018). Our
462 results indicate that environmental variation, beyond the territory and year effects modelled in
463 this study, explains most of the variation in telomere length in the Seychelles warbler, and
464 indicates a low potential for telomere length to respond to selection.

465

466 The previous studies investigating telomere heritability in wild populations using an animal
467 model approach, which separated out some confounding effects, found differing results

regarding the contribution of environmental factors to telomere variation. In the white-throated dipper *Cinclus cinclus*, heritability was not significant, but there were strong nest (0.20 ± 0.08 SE) and year of birth effects (0.46 ± 0.13 SE) on telomere length variation (Becker et al. 2015). In comparison, in the great reed warbler *Acrocephalus arundinaceus*, high heritability (0.48 ± 0.12 SE) and equally large maternal effects (0.47 ± 0.09 SE) appeared to underlie telomere variation (Asghar et al. 2015). In our study, if we do not account for shared environment effects heritability was 0.080 (95% CrI: 0.041-0.144) and 0.048 (95% CrI: <0.001-0.087) after accounting for natal and current environmental effects, technical effects and parental effects. Further, despite the number of environmental factors measured, including cohort, season, territory and parental effects, our final model only provided evidence for small effects of current season on telomere length variation (0.036, 95% CrI: 0.018-0.101). This contrasts with a number of studies which have observed higher telomere loss in poor natal environment cohorts (Boonekamp et al. 2014; Watson et al. 2015), or suggest an impact of cohort or maternal effects on telomere variation (Asghar et al. 2015; Becker et al. 2015; Fairlie et al. 2016). The lack of parental effects in the Seychelles warbler population may have been caused by the high levels of extra-pair paternity or cobreeding by subordinate females (Richardson et al. 2001; Raj Pant et al. 2019), which would result in parental care being provided by the social rather than genetic parent. However, including the social rather than genetic parents in the model did not provide evidence for parental effects. Finally, the lack of natal/parental effects may be because these are only apparent early in life and are diluted when looking at lifelong telomere measures. Indeed, previously we have found cohort effects on juvenile telomere length that did not extend to measures beyond the natal year (Spurgin et al. 2018).

The lack of parental or early life environment effects in our study may also reflect the sampling regime of our population whereby only a small proportion of samples in the dataset were

493 measured as nestlings (12% chicks). Due to the inaccessibility of nests, which may be up to 30
494 metres up in trees, individuals are usually caught as fledglings during their first 3 months when
495 they remain dependent on their parents (Komdeur 1994; Brouwer et al. 2006). While early-life
496 measures of telomeres will be closer to the inherited telomere length, by using a measure of
497 telomere length across the lifetimes of birds we are measuring a product of inheritance, attrition
498 and restoration/lengthening. After birth, telomere attrition occurs rapidly (Hall et al. 2004;
499 Salomons et al. 2009) and telomere length decreases with age quickest in the first few weeks of
500 life in the Seychelles warbler (Spurgin et al. 2018). With more samples from younger or older
501 individuals it would be possible to investigate how different genetic and environmental effects
502 contribute to telomere variation at different time points. Further, we could have tested for genetic
503 correlations between telomere measures in early and late life and investigated the presence of
504 genotype-by-age interactions. However, our power to calculate heritabilities were lower using
505 measures taken only as nestlings (N = 324 measures of 319 birds, power $\geq$ 0.80 to detect
506 heritabilities of $\geq$ 0.23) or individuals showing senescent declines in reproduction and survival
507 (>7 years (Hammers et al. 2015), N = 249 measures of 161 birds, power $\geq$ 0.80 to detect
508 heritabilities of $\geq$ 0.40). It is likely that the measurement of telomeres in nestlings in previous
509 studies has resulted in higher heritability, or larger maternal or cohort effects due to the use of
510 only very early-life telomere measures (Asghar et al. 2015; Becker et al. 2015). Further studies
511 investigating how additive genetic variance in telomere length changes with age, and
512 investigating genetic correlations between early and late life, are warranted to understand the
513 genetic constraints on relative telomere length (Dugdale and Richardson 2018).

514

515 An important finding from our study is the impact of technical variation on telomere length
516 measurements. Storage time did not affect telomere length (Spurgin et al. 2018). In contrast, the
517 technician handling the qPCR did have an effect on RTL estimates, and we did find considerable

plate effects. While the golden sample should standardise samples within a plate to minimise plate variation it is clear that running the golden sample in a few wells is not capturing differences between plates completely resulting in between plate variation. Previous studies estimating heritability of telomere length using animal models have not included experimental effects likely due to small sample sizes. This technical variance has the potential to bias heritabilities if not included in the analyses (Ponzi et al. 2018). Heritabilities can be re-evaluated with the total phenotypic variance excluding any technical variance to reflect true biological variance (de Villemereuil et al. 2018). Measurement error in qPCR studies could come from various factors such as between- and within-plate effects, technician, storage time, changes in reagents and extraction method effects (Eisenberg et al. 2015; Seeker et al. 2016; Reichert et al. 2017; van Lieshout et al. 2020*b*). Such measurement error should be incorporated into analyses and reported to prevent it from biasing results (Nettle et al. 2019). Further, future studies should ensure samples on plates reflect multiple years and ages to enable the greatest statistical power to separate variances of technical and biological interest (van Lieshout et al. 2020*b*).

In conclusion, our results illustrate that heritability of telomere length would not be identified from parent–offspring regression analyses, and only by using more complex quantitative genetic models could a reliable heritability estimate be calculated. In our population, telomere length variation across an individual's lifetime was largely driven by environmental factors, including a small catch season effect. There was evidence for a negative, but weak, within-paternal age at conception effect and a positive but weak among-maternal age at conception effect. Further work is needed to see how heritability estimates of telomere length, and telomere loss, calculated using the appropriate power and analytical tools, compare across wild populations.

Acknowledgements

We thank the Department of Environment and Seychelles Bureau of Standards for research permits and Nature Seychelles for the opportunity to conduct fieldwork on Cousin Island. We thank the many field volunteers and all those who have contributed to long-term data collection in the Seychelles warbler project, Owen Howison for maintenance of the Seychelles warbler database and Marco van der Velde for microsatellite genotyping. Part of these analyses were undertaken on ARC3, part of the High Performance Computing facilities at the University of Leeds, UK as well as sharc and iceberg, part of the High Performance Computing facilities at the University of Sheffield, UK. This work was supported by NERC grant (NE/B504106/1 to T.A.B. and D.S.R.; H.L.D. was postdoc on this), NWO Rubicon (825.09.013) and NERC (NE/I021748/1) fellowships to H.L.D., Lucie Burgers Foundation and KNAW Schure Beijerinck Poppings grant (SBP2013/04) to H.L.D., NWO visitors grant (040.11.232 to J.K. and H.L.D.), NERC grant (NE/P011284/1 to H.L.D. and D.S.R.; A.M.S. was postdoc on this), NWO grants (854.11.003 and 823.01.014 to J.K.) and NERC grants (NE/F02083X/1 and NE/K005502/1 to D.S.R.).

References

Asghar, M., S. Bensch, M. Tarka, B. Hansson, and D. Hasselquist. 2015. Maternal and genetic factors determine early life telomere length. Proceedings of the Royal Society B: Biological Sciences 282:20142263.

Aston, K. I., S. C. Hunt, E. Susser, M. Kimura, P. Factor-Litvak, D. Carrell, and A. Aviv. 2012. Divergence of sperm and leukocyte age-dependent telomere dynamics: implications for male-driven evolution of telomere length in humans. Molecular Human Reproduction 18:517–522.

Aubert, G., G. M. Baerlocher, I. Vulto, S. S. Poon, and P. M. Lansdorp. 2012. Collapse of telomere homeostasis in hematopoietic cells caused by heterozygous mutations in telomerase genes. PLoS Genetics 8:e1002696.

Aviv, A., and E. Susser. 2013. Leukocyte telomere length and the father's age enigma: implications for population health and for life course. International Journal of Epidemiology 42:457–462.

Barrett, E. L. B., T. A. 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.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Bauch, C., P. H. Becker, and S. Verhulst. 2012. Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. Proceedings of the Royal Society B: Biological Sciences 280:20122540–20122540.

Bauch, C., J. J. Boonekamp, P. Korsten, E. Mulder, and S. Verhulst. 2019. Epigenetic inheritance of telomere length in wild birds. PLOS Genetics 15:e1007827.

Bebbington, K., S. A. Kingma, E. A. Fairfield, H. L. Dugdale, J. Komdeur, L. G. Spurgin, and D. S. Richardson. 2017. Kinship and familiarity mitigate costs of social conflict between Seychelles warbler neighbors. Proceedings of the National Academy of Sciences 114:E9036–E9045.

Bebbington, K., L. G. Spurgin, E. A. Fairfield, H. L. Dugdale, J. Komdeur, T. Burke, and D. S. Richardson. 2016. Telomere length reveals cumulative individual and transgenerational inbreeding effects in a passerine bird. Molecular Ecology 25:2949–2960.

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 282:20142924–20142924.

Belmaker, A., K. K. Hallinger, R. A. Glynn, D. W. Winkler, and M. F. Haussmann. 2019. The environmental and genetic determinants of chick telomere length in Tree Swallows (*Tachycineta bicolor*). Ecology and Evolution 9:8175–8186.

Bijma, P. 2011. A general definition of the heritable variation that determines the potential of a population to respond to selection. Genetics 189:1347–1359.

Bize, P., F. Criscuolo, N. B. Metcalfe, L. Nasir, and P. Monaghan. 2009. Telomere dynamics rather than age predict life expectancy in the wild. Proceedings of the Royal Society B: Biological Sciences 276:1679–1683.

601 Blackburn, E. H. 1991. Structure and function of telomeres. Nature 350:569–573.

602 Blackburn, E. H., E. S. Epel, and J. Lin. 2015. Human telomere biology: A contributory and
603 interactive factor in aging, disease risks, and protection. Science 350:1193–1198.

604 Boonekamp, J. J., G. A. Mulder, H. M. Salomons, C. Dijkstra, and S. Verhulst. 2014. Nestling
605 telomere shortening, but not telomere length, reflects developmental stress and predicts survival
606 in wild birds. Proceedings of the Royal Society B: Biological Sciences 281:20133287–
607 20133287.

608 Boonekamp, J. J., M. J. P. Simons, L. Hemerik, and S. Verhulst. 2013. Telomere length behaves
609 as biomarker of somatic redundancy rather than biological age. Aging Cell 12:330–332.

610 Bouwhuis, S., S. Verhulst, C. Bauch, and O. Vedder. 2018. Reduced telomere length in offspring
611 of old fathers in a long-lived seabird. Biology Letters 14:20180213.

612 Broer, L., V. Codd, D. R. Nyholt, J. Deelen, M. Mangino, G. Willemsen, E. Albrecht, N. Amin,
613 M. Beekman, E. J. C. de Geus, A. Henders, C. P. Nelson, C. J. Steves, M. J. Wright, A. J. M. de
614 Craen, A. Isaacs, M. Matthews, A. Moayyeri, G. W. Montgomery, B. A. Oostra, J. M. Vink, T.
615 D. Spector, P. E. Slagboom, N. G. Martin, N. J. Samani, C. M. van Duijn, and D. I. Boomsma.
616 2013. Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger
617 maternal inheritance and a paternal age effect. European Journal of Human Genetics 21:1163–
618 1168.

619 Brouwer, L., I. Barr, M. Van De Pol, T. Burke, J. Komdeur, and D. S. Richardson. 2010. MHC-
620 dependent survival in a wild population: evidence for hidden genetic benefits gained through
621 extra-pair fertilizations. Molecular Ecology 19:3444–3455.

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

625 Butler, D. G., B. R. Cullis, A. R. Gilmour, and B. J. Gogel. 2009. Mixed models for S language
626 environments, ASReml-R reference manual. Queensland Department of Primary Industries and
627 Fisheries, Brisbane.

628 Campisi, J. 2005. Senescent cells, tumor suppression, and organismal aging: good citizens, bad
629 neighbors. Cell 120:513–522.

630 Cesare, A. J., and R. R. Reddel. 2010. Alternative lengthening of telomeres: models, mechanisms
631 and implications. Nature Reviews Genetics 11:319–330.

632 Champely, S. 2018. pwr: basic functons for power analysis. R package version 1.2-2
633 https://CRAN.R-project.org/package=pwr.

634 Charmantier, A., J. E. Brommer, and D. H. Nussey. 2014. The quantitative genetics of
635 senescence in wild animals. Pages 68–83 *in*Quantitative Genetics in the Wild. Oxford University
636 Press, Oxford.

637 Criscuolo, F., S. Zahn, and P. Bize. 2017. Offspring telomere length in the long lived Alpine
638 swift is negatively related to the age of their biological father and foster mother. Biology Letters
639 13:20170188.

640 de Frutos, C., A. P. López-Cardona, N. Fonseca Balvís, R. Laguna-Barraza, D. Rizos, A.
641 Gutierrez-Adán, and P. Bermejo-Álvarez. 2016. Spermatozoa telomeres determine telomere
642 length in early embryos and offspring. Reproduction 151:1–7.

De Meyer, T., E. R. Rietzschel, M. L. De Buyzere, D. De Bacquer, W. Van Criekinge, G. G. De Backer, T. C. Gillebert, P. Van Oostveldt, and S. Bekaert. 2007. Paternal age at birth is an important determinant of offspring telomere length. Human Molecular Genetics 16:3097–3102.

de Villemereuil, P., M. B. Morrissey, S. Nakagawa, and H. Schielzeth. 2018. Fixed-effect variance and the estimation of repeatabilities and heritabilities: issues and solutions. Journal of Evolutionary Biology 31:621–632.

Delgado, D. A., C. Zhang, K. Gleason, K. Demanelis, L. S. Chen, J. Gao, S. Roy, J. Shinkle, M. Sabarinathan, M. Argos, L. Tong, A. Ahmed, T. Islam, M. Rakibuz-Zaman, G. Sarwar, H. Shahriar, M. Rahman, M. Yunus, J. A. Doherty, F. Jasmine, M. G. Kibriya, H. Ahsan, and B. L. Pierce. 2019. The contribution of parent-to-offspring transmission of telomeres to the heritability of telomere length in humans. Human Genetics 138:49–60.

Dugdale, H. L., and D. S. Richardson. 2018. Heritability of telomere variation: it is all about the environment! Philosophical Transactions of the Royal Society B: Biological Sciences 373:20160450.

Dupont, S. M., C. Barbraud, O. Chastel, K. Delord, S. Ruault, H. Weimerskirch, and F. Angelier. 2018. Young parents produce offspring with short telomeres: A study in a long-lived bird, the Black-browed Albatross (*Thalassarche melanophrys)*. PLoS ONE 13:e0193526.

Edwards, H. A., T. Burke, and H. L. Dugdale. 2017. Repeatable and heritable behavioural variation in a wild cooperative breeder. Behavioral Ecology 28:668–676.

Eisenberg, D. T. A. 2019. Paternal age at conception effects on offspring telomere length across species—What explains the variability? PLOS Genetics 15:e1007946.

Eisenberg, D. T. A., M. G. Hayes, and C. W. Kuzawa. 2012. Delayed paternal age of reproduction in humans is associated with longer telomeres across two generations of descendants. Proceedings of the National Academy of Sciences 109:10251–10256.

Eisenberg, D. T. A., and C. W. Kuzawa. 2018. The paternal age at conception effect on offspring telomere length: mechanistic, comparative and adaptive perspectives. Philosophical Transactions of the Royal Society B: Biological Sciences 373:20160442.

Eisenberg, D. T. A., C. W. Kuzawa, and M. G. Hayes. 2015. Improving qPCR telomere length assays: Controlling for well position effects increases statistical power. American Journal of Human Biology 27:570–575.

Eisenberg, D. T. A., J. Tackney, R. M. Cawthon, C. T. Cloutier, and K. Hawkes. 2017. Paternal and grandpaternal ages at conception and descendant telomere lengths in chimpanzees and humans. American Journal of Physical Anthropology 162:201–207.

Fairlie, J., R. Holland, J. G. Pilkington, J. M. Pemberton, L. Harrington, and D. H. Nussey. 2016. Lifelong leukocyte telomere dynamics and survival in a free-living mammal. Aging Cell 15:140–148.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics (4th Ed.). Pearson Prentice Hall, Essex.

Foley, N. M., E. J. Petit, T. Brazier, J. A. Finarelli, G. M. Hughes, F. Touzalin, S. J. Puechmaille, and E. C. Teeling. 2020. Drivers of longitudinal telomere dynamics in a long-lived bat species, *Myotis myotis*. Molecular Ecology 00:1–5.

Froy, H., E. J. Bird, R. V. Wilbourn, J. Fairlie, S. L. Underwood, E. Salvo-Chirnside, J. G.

685 Pilkington, C. Bérénos, J. M. Pemberton, and D. H. Nussey. 2017. No evidence for parental age
686 effects on offspring leukocyte telomere length in free-living Soay sheep. Scientific Reports
687 7:9991.

688 Green, P., and C. J. MacLeod. 2016. SIMR: an R package for power analysis of generalized
689 linear mixed models by simulation. Methods in Ecology and Evolution 7:493–498.

690 Greider, C. W., and E. H. Blackburn. 1989. A telomeric sequence in the RNA of *Tetrahymena*
691 telomerase required for telomere repeat synthesis. Nature 337:331–337.

692 Hadfield, J. D. 2010. MCMC methods for multi-response generalized Linear mixed models: The
693 MCMCglmm R package. Journal of Statistical Software 33:1–22.

694 ———. 2019. MCMCglmm Course Notes.

695 Hadfield, J. D., D. S. Richardson, and T. Burke. 2006. Towards unbiased parentage assignment:
696 combining genetic, behavioural and spatial data in a Bayesian framework. Molecular Ecology
697 15:3715–3730.

698 Hall, M. E., L. Nasir, F. Daunt, E. A. Gault, J. P. Croxall, S. Wanless, and P. Monaghan. 2004.
699 Telomere loss in relation to age and early environment in long-lived birds. Proceedings of the
700 Royal Society of London. Series B: Biological Sciences 271:1571–1576.

701 Hammers, M., S. A. Kingma, K. Bebbington, J. van de Crommenacker, L. G. Spurgin, D. S.
702 Richardson, T. Burke, H. L. Dugdale, and J. Komdeur. 2015. Senescence in the wild: Insights
703 from a long-term study on Seychelles warblers. Experimental Gerontology 71:69–79.

704 Hammers, M., S. A. Kingma, L. G. Spurgin, K. Bebbington, H. L. Dugdale, T. Burke, J.
705 Komdeur, and D. S. Richardson. 2019. Breeders that receive help age more slowly in a
706 cooperatively breeding bird. Nature Communications 10:1301.

707 Hammers, M., D. S. Richardson, T. Burke, and J. Komdeur. 2012. Age-dependent terminal
708 declines in reproductive output in a wild bird. PLoS ONE 7:e40413.

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

712 Harley, C. B., H. Vaziri, C. M. Counter, and R. C. Allsopp. 1992. The telomere hypothesis of
713 cellular aging. Experimental Gerontology 27:375–382.

714 Heidinger, B. J., J. D. Blount, W. Boner, K. Griffiths, N. B. Metcalfe, and P. Monaghan. 2012.
715 Telomere length in early life predicts lifespan. Proceedings of the National Academy of Sciences
716 109:1743–1748.

717 Heidinger, B. J., K. A. Herborn, H. M. V. Granroth-Wilding, W. Boner, S. Burthe, M. Newell, S.
718 Wanless, F. Daunt, and P. Monaghan. 2016. Parental age influences offspring telomere loss.
719 Functional Ecology 30:1531–1538.

720 Hjelmborg, J. B., C. Dalgård, M. Mangino, T. D. Spector, U. Halekoh, S. Möller, M. Kimura, K.
721 Horvath, J. D. Kark, K. Christensen, K. O. Kyvik, and A. Aviv. 2015. Paternal age and telomere
722 length in twins: the germ stem cell selection paradigm. Aging Cell 14:701–703.

723 Kimura, M., L. F. Cherkas, B. S. Kato, S. Demissie, J. B. Hjelmborg, M. Brimacombe, A.
724 Cupples, J. L. Hunkin, J. P. Gardner, X. Lu, X. Cao, M. Sastrasinh, M. A. Province, S. C. Hunt,
725 K. Christensen, D. Levy, T. D. Spector, and A. Aviv. 2008. Offspring's leukocyte telomere

length, paternal age, and telomere elongation in sperm. PLoS Genetics 4:e37.

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

———. 1994. Experimental evidence for helping and hindering by previous offspring in the cooperative-breeding Seychelles warbler *Acrocephalus sechellensis*. Behavioral Ecology and Sociobiology 34:175–186.

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. Bird Conservation International 1:177–185.

Komdeur, J., and S. Daan. 2005. Breeding in the monsoon: semi-annual reproduction in the Seychelles warbler (*Acrocephalus sechellensis*). Journal of Ornithology 146:305–313.

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.

Kruuk, L. E. B. 2004. Estimating genetic parameters in natural populations using the "animal model." Philosophical Transactions of the Royal Society B: Biological Sciences 359:873–890.

Kruuk, L. E. B., and J. D. Hadfield. 2007. How to separate genetic and environmental causes of similarity between relatives. Journal of Evolutionary Biology 20:1890–1903.

Kruuk, L. E. B., J. Slate, and A. J. Wilson. 2008. New answers for old questions: the evolutionary quantitative genetics of wild animal populations. Annual Review of Ecology, Evolution, and Systematics 39:525–548.

Levy, M. Z., R. C. Allsopp, A. B. Futcher, C. W. Greider, and C. B. Harley. 1992. Telomere end-replication problem and cell aging. Journal of Molecular Biology 225:951–960.

López-Otín, C., M. A. Blasco, L. Partridge, M. Serrano, and G. Kroemer. 2013. The hallmarks of aging. Cell 153:1194–1217.

Lynch, M., and B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. Sinauer, Sunderland, MA.

McLennan, D., J. D. Armstrong, D. C. Stewart, S. McKelvey, W. Boner, P. Monaghan, and N. B. Metcalfe. 2018. Links between parental life histories of wild salmon and the telomere lengths of their offspring. Molecular Ecology 27:804–814.

Morrissey, M. B., and A. J. Wilson. 2010. pedantics: an r package for pedigree-based genetic simulation and pedigree manipulation, characterization and viewing. Molecular Ecology Resources 10:711–719.

Nakagawa, S., N. J. Gemmell, and T. Burke. 2004. Measuring vertebrate telomeres: applications and limitations. Molecular Ecology 13:2523–2533.

Nettle, D., L. Seeker, D. Nussey, H. Froy, and M. Bateson. 2019. Consequences of measurement error in qPCR telomere data: A simulation study. PLOS ONE 14:e0216118.

Noguera, J. C., N. B. Metcalfe, and P. Monaghan. 2018. Experimental demonstration that offspring fathered by old males have shorter telomeres and reduced lifespans. Proceedings of the Royal Society B: Biological Sciences 285:20180268.

Nordfjäll, K., U. Svenson, K.-F. Norrback, R. Adolfsson, and G. Roos. 2010. Large-scale parent-child comparison confirms a strong paternal influence on telomere length. European Journal of Human Genetics 18:385–389.

Nussey, D. H., T. Coulson, M. Festa-Bianchet, and J.-M. Gaillard. 2008. Measuring senescence in wild animal populations: towards a longitudinal approach. Functional Ecology 22:393–406.

Olsson, M., A. Pauliny, E. Wapstra, T. Uller, T. Schwartz, and D. Blomqvist. 2011. Sex differences in sand lizard telomere inheritance: Paternal epigenetic effects increases telomere heritability and offspring survival. PLoS ONE 6:e17473.

Ponzi, E., L. F. Keller, T. Bonnet, and S. Muff. 2018. Heritability, selection, and the response to selection in the presence of phenotypic measurement error: Effects, cures, and the role of repeated measurements. Evolution 72:1992–2004.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raj Pant, S., J. Komdeur, T. Burke, H. L. Dugdale, and D. S. Richardson. 2019. Socio-ecological conditions and female infidelity in the Seychelles warbler. Behavioral Ecology 30:1254–1264.

Reichert, S., H. Froy, W. Boner, T. M. Burg, F. Daunt, R. Gillespie, K. Griffiths, S. Lewis, R. A. Phillips, D. H. Nussey, and P. Monaghan. 2017. Telomere length measurement by qPCR in birds is affected by storage method of blood samples. Oecologia 184:341–350.

Reichert, S., E. R. Rojas, S. Zahn, J.-P. Robin, F. Criscuolo, and S. Massemin. 2015. Maternal telomere length inheritance in the king penguin. Heredity 114:10–16.

Richardson, D. S., T. Burke, and J. Komdeur. 2007. Grandparent helpers: the adaptive significance of older, postdominant helpers in the Seychelles warbler. Evolution 61:2790–2800.

Richardson, D. S., F. L. Jury, K. Blaakmeer, J. Komdeur, and T. Burke. 2001. Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). Molecular Ecology 10:2263–2273.

Salomons, H. M., G. A. Mulder, L. van de Zande, M. F. Haussmann, M. H. K. Linskens, and S. Verhulst. 2009. Telomere shortening and survival in free-living corvids. Proceedings of the Royal Society B: Biological Sciences 276:3157–3165.

Seeker, L. A., R. Holland, S. Underwood, J. Fairlie, A. Psifidi, J. J. Ilska, A. Bagnall, B. Whitelaw, M. Coffey, G. Banos, and D. H. Nussey. 2016. Method specific calibration corrects for DNA extraction method effects on relative telomere length measurements by quantitative PCR. PLOS ONE 11:e0164046.

Simons, M. J. P. 2015. Questioning causal involvement of telomeres in aging. Ageing Research Reviews 24:191–196.

Spurgin, L. G., K. Bebbington, E. A. Fairfield, M. Hammers, J. Komdeur, T. Burke, H. L. Dugdale, and D. S. Richardson. 2018. Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. Journal of Animal Ecology 87:187–198.

Unryn, B. M., L. S. Cook, and K. T. Riabowol. 2005. Paternal age is positively linked to telomere length of children. Aging Cell 4:97–101.

van de Pol, M., and S. Verhulst. 2006. Age-Ddependent traits: A new statistical model to separate within- and between-individual effects. The American Naturalist 167:766–773.

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

van Lieshout, S. H. ., A. M. Sparks, A. Bretman, C. Newman, C. D. Buesching, T. Burke, D. W. Macdonald, and H. L. Dugdale. 2020*a*. Estimation of environmental and genetic contributions to telomere length variation in a wild mammal. EcoEvoRxiv March 4:doi:10.32942/osf.io/s5dnr.

van Lieshout, S. H. J., H. Froy, J. Schroeder, T. Burke, M. J. P. Simons, and H. L. Dugdale. 2020*b*. Slicing: A sustainable approach to structuring samples for analysis in long-term studies. Methods in Ecology and Evolution 11:418–430.

Verhulst, S. 2020. Improving comparability between qPCR-based telomere studies. Molecular Ecology Resources 20:11–13.

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. Journal of Experimental Biology 218:668–674.

Wilbourn, R. V., J. P. Moatt, H. Froy, C. A. Walling, D. H. Nussey, and J. J. Boonekamp. 2018. The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. Philosophical Transactions of the Royal Society B: Biological Sciences 373:20160447.

Wilson, A. J. 2008. Why h2 does not always equal VA/VP? Journal of Evolutionary Biology 21:647–650.

Wilson, A. J., D. Réale, M. N. Clements, M. M. Morrissey, E. Postma, C. A. Walling, L. E. B. Kruuk, and D. H. Nussey. 2010. An ecologist's guide to the animal model. Journal of Animal Ecology 79:13–26.

Young, A. J. 2018. The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. Philosophical Transactions of the Royal Society B: Biological Sciences 373:20160452.

Data Accessibility

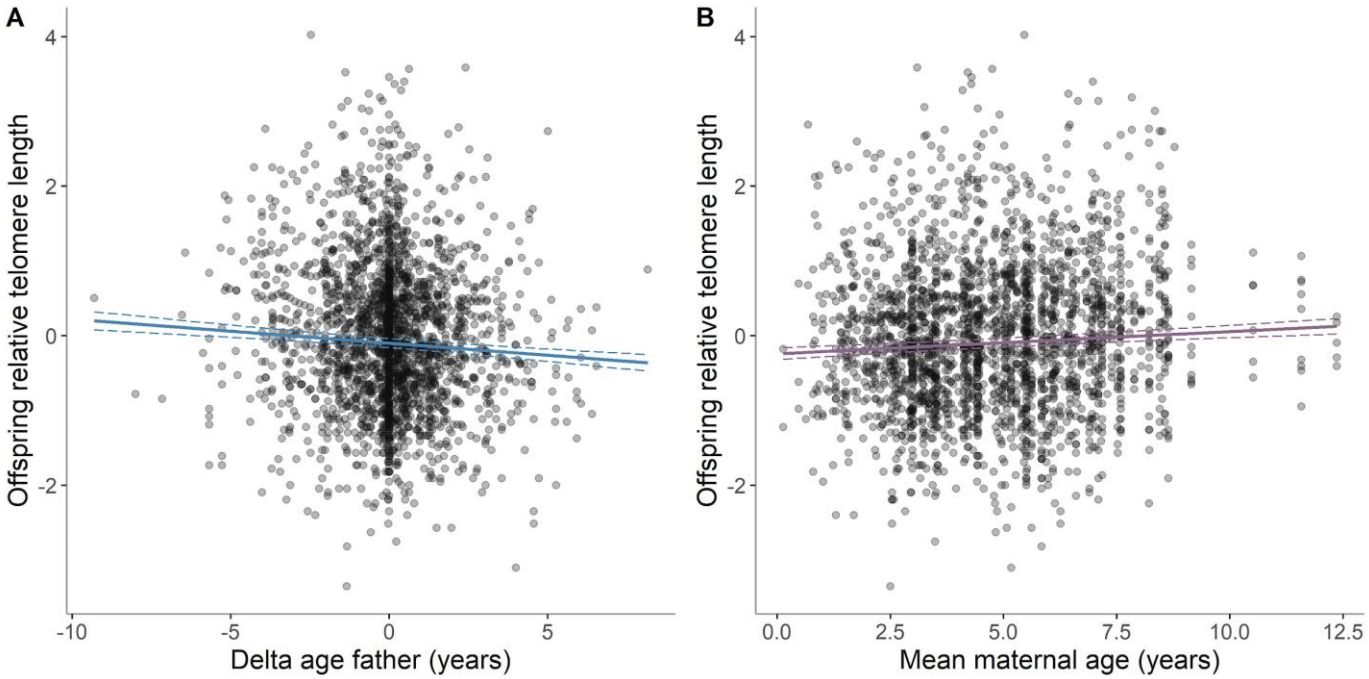
Data will be deposited in the Dryad Digital Repository upon acceptance.

Author Contributions

This study was conceived by H.L.D. and D.S.R. and developed by A.M.S. D.S.R., H.L.D., T.B. and J.K. manage the long-term Seychelles warbler study system. Samples were collected by D.S.R., K.B., H.L.D. and T.B. Molecular work was undertaken by M.V., E.A.F., K.B., L.G.S. and D.S.R. The genetic pedigree was constructed by H.L.D. A.M.S. performed the statistical analyses and wrote the first draft of the manuscript with input from H.L.D. and D.S.R. All authors provided comments on the manuscript and gave final approval for publication.

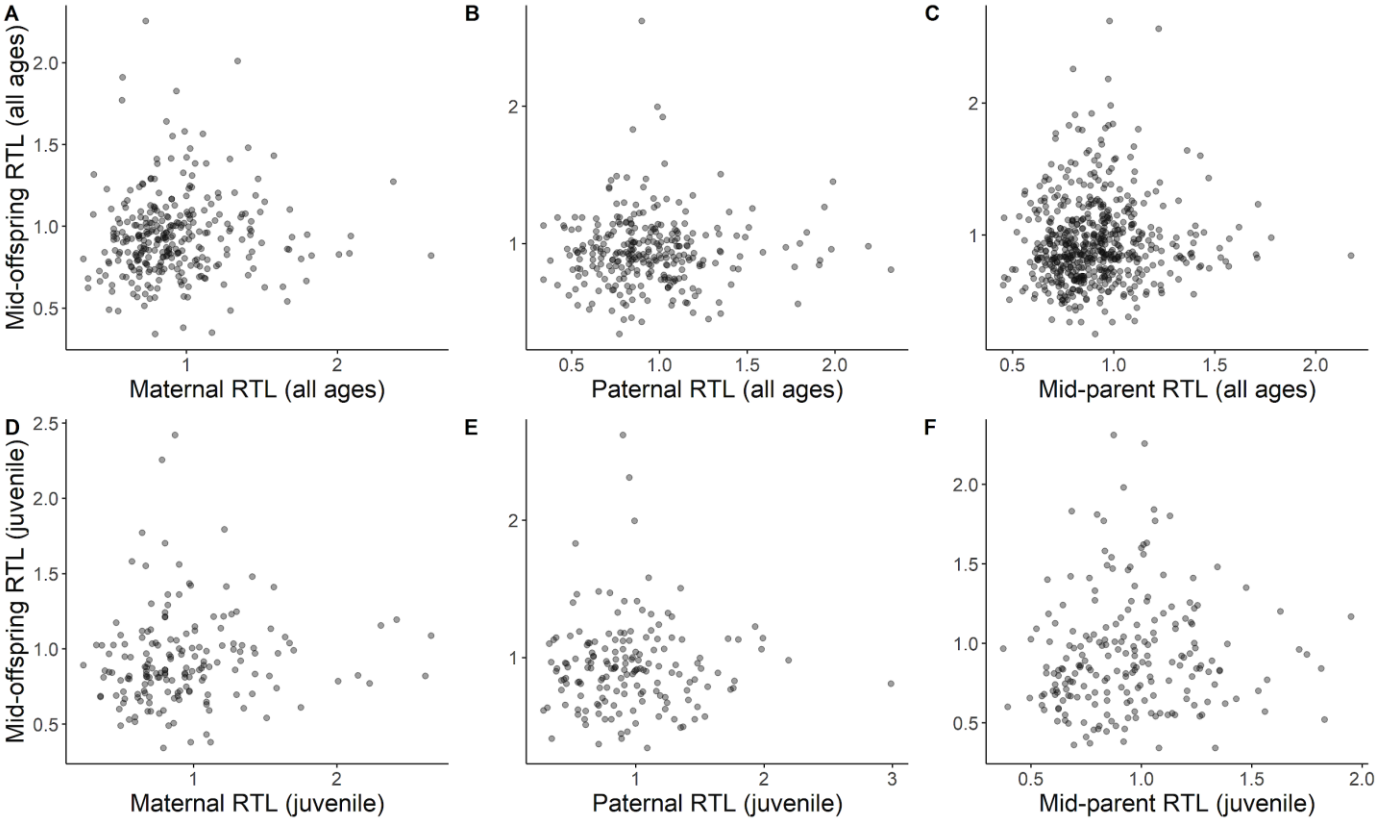
Table 1. Linear mixed model results investigating between versus within maternal and paternal age at conception (MAC and PAC, respectively) effects on offspring telomere length in the Seychelles warbler using the within-subject centering method (van de Pol and Wright 2009). Associations were investigated in offspring telomere length of all ages (2361 RTL measures of 1156 offspring) and included are the estimated effects (estimate), standard errors (SEs), and significance of fixed effects based on a likelihood ratio test (LRT; P-value) where df=1. Relative telomere length was square-root then z-transformed in both models and age was log-transformed. Model 1 investigates within-MAC/PAC effects (deviation from the mean age at conception of the parent: DevMeanMAC/PAC) and between-MAC/PAC (mean age at conception for each parent: meanMAC/PAC) effects. Model 2 investigates whether these within and between slopes are significantly different from each other (mean MAC/PAC representing the difference between the slopes and MAC/PAC which becomes the within-MAC/PAC slope identical to Model 1). P<0.05 are shown in bold.

	Model 1				Model 2			
variables	estimate	SE	LRT	P-value	estimate	SE	LRT	P-value
fixed effects								
Intercept	-0.302	0.092			-0.302	0.092		
Log Age (years)	**-0.311**	**0.030**	**102.250**	**<0.001**	**-0.311**	**0.030**	**102.250**	**<0.001**
Sex (male)	0.024	0.039	0.374	0.541	0.024	0.039	0.374	0.541
Technician	**0.465**	**0.076**	**35.954**	**<0.001**	**0.465**	**0.076**	**35.954**	**<0.001**
MeanMAC	**0.030**	**0.011**	**7.482**	**0.006**	**0.039**	**0.016**	**6.002**	**0.014**
DevMeanMAC	-0.010	0.011	0.726	0.394				
MAC					-0.010	0.011	0.726	0.394
MeanPAC	0.001	0.009	0.011	0.918	**0.033**	**0.015**	**5.102**	**0.024**
DevMeanPAC	**-0.032**	**0.011**	**8.032**	**0.005**				
PAC					**-0.032**	**0.011**	**8.032**	**0.005**
random effects								
ID	0.037				0.037			
Mother identity	0.023				0.023			
Father identity	0.005				0.005			
Plate ID	0.186				0.186			
Season ID	0.030				0.030			
Residual	0.653				0.653			



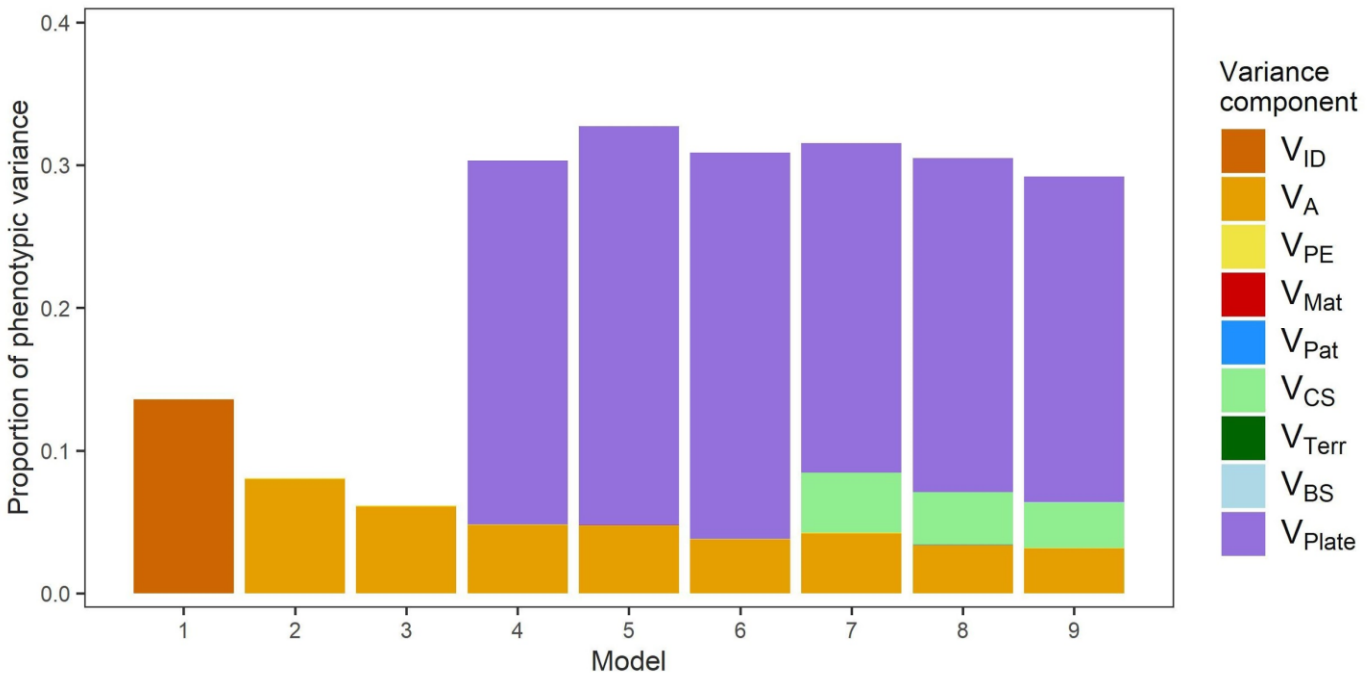
862
863 **Figure 1.** Scatterplots of raw relative telomere length data from the Seychelles warbler showing
864 significant negative within-paternal age at conception effects (A) and positive between maternal
865 age at conception effects (B) on offspring telomere length across all ages (2361 RTL measures of
866 1156 offspring). Lines indicate mixed model predictions (Model 1, Table 1) using a within-
867 subject centering method with dashed lines indicating standard errors. Data points are semi-
868 transparent to show overlapping values.

869



870

871 **Figure 2.** Mid-offspring relative telomere length (RTL) in relation to their mother's (A, D),
872 father's (B, E) or mid-parent (C, F) RTL in the Seychelles warbler. Data are presented with mean
873 RTL measures across all ages for both offspring and parents (A-C) and for mean juvenile (<1
874 year) measures of both offspring and parents (D-F). Where parents had multiple offspring, a
875 mean of the mean RTL from their offspring was taken. Full model results are provided in Table
876 S4 and sample sizes are 303 (A), 284 (B), 585 (C), 172 (D), 165 (E) and 210 (F).



877

878 **Figure 3.** Estimated variance components as proportions of total phenotypic variance in relative
879 telomere length determined using univariate models in the Seychelles warbler. Models were
880 fitted additively with increasing random or fixed effects as follows: Model 1 – individual identity
881 (V_{ID}), 2 – partitioning of V_{ID} into additive genetic (V_A) and permanent environment (V_{PE})
882 components, 3 – the addition of fixed effects (age, sex, technician), 4 – qPCR plate ID (V_{Plate}), 5
883 – maternal identity (V_{Mat}), 6 – paternal identity (V_{Pat}), 7 – capture season (V_{CS}), 8 – current
884 territory (V_{Terr}) and 9 – birth season (V_{BS}). For full model results see Table 2 and S6.

885

Table 2. Animal model variance component estimates and their associated proportions of the phenotypic variance from a MCMC model of relative telomere length in the Seychelles warbler. Results are from model 9, the model with all variance components and fixed effects estimated (see Methods). Variance components reported are the: additive genetic (V_A), permanent environment (V_{PE}), qPCR plate (V_{Plate}), maternal identity (V_{Mat}), paternal identity (V_{Pat}), capture season (V_{CS}), current territory (V_{CTerr}), birth season (V_{BS}), and residual (V_R) variance. Included are the variance component estimates as the posterior mode along with their lower and upper 95% credible intervals (CrI) and the proportion of the total phenotypic variance explained by the term (Prop V_P) with their associated 95% CrI. Significance of fixed effects were determined by whether the 95% CrI did not overlap zero (shown in bold).

Variables	Posterior mode	Lower 95% CrI	Upper 95% CrI	Prop V_P	Lower 95% CrI	Upper 95% CrI
Random effects						
V_A	0.005	<0.001	0.010	0.031	<0.001	0.067
V_{PE}	<0.001	<0.001	0.008	<0.001	<0.001	0.053
V_{Plate}	0.035	0.026	0.045	0.228	0.186	0.287
V_{Mat}	<0.001	<0.001	0.003	<0.001	<0.001	0.022
V_{Pat}	<0.001	<0.001	0.002	<0.001	<0.001	0.011
V_{CS}	0.005	0.002	0.012	0.032	0.013	0.079
V_{Terr}	<0.001	<0.001	0.003	<0.001	<0.001	0.017
V_{BS}	<0.001	<0.001	0.003	<0.001	<0.001	0.021
V_R	0.096	0.090	0.104	0.635	0.588	0.703
Fixed effects						
Intercept	0.864	0.816	0.919			
Sex (male)	0.014	-0.017	0.036	-	-	-
Log Age (years)	**-0.117**	**-0.142**	**-0.096**	-	-	-
Technician	**0.199**	**0.129**	**0.245**	-	-	-

Table 3. Animal model variance component estimates and their associated proportions from a MCMC model of relative telomere length (RTL) in the Seychelles warbler comparing parental effects where genetic parents are included (left, Model 7) and social parents are included (right, Model 10). Variance components reported are the: additive genetic (V_A), permanent environment (V_{PE}), qPCR plate (V_{Plate}), maternal identity (V_{Mat}), paternal identity (V_{Pat}), capture season (V_{CS}), and residual (V_R) variance. Variance component estimates are reported as the posterior mode along with their 95% credible intervals (Lower 95% CrI, Upper 95% CrI) and the proportion of the total phenotypic variance explained by the term (Prop V_P) with their associated 95% credible intervals.

	Model with genetic parents						**Model with social parents**					
Random effects	Posterior mode	Lower 95% CrI	Upper 95% CrI	Prop V_P	Lower 95% CrI	Upper 95% CrI	Posterior mode	Lower 95% CrI	Upper 95% CrI	Prop V_P	Lower 95% CrI	Upper 95% CrI
V_A	0.006	0.002	0.011	0.042	0.012	0.075	0.007	0.001	0.011	0.042	0.008	0.073
V_{PE}	<0.001	<0.001	0.009	<0.001	<0.001	0.059	<0.001	<0.001	0.009	<0.001	<0.001	0.056
V_{Plate}	0.037	0.028	0.047	0.231	0.193	0.295	0.034	0.028	0.047	0.233	0.193	0.294
V_{Mat}	<0.001	<0.001	0.004	<0.001	<0.001	0.024	<0.001	<0.001	0.003	<0.001	<0.001	0.017
V_{Pat}	<0.001	<0.001	0.002	<0.001	<0.001	0.011	<0.001	<0.001	0.002	<0.001	<0.001	0.016
V_{CS}	0.004	0.002	0.012	0.042	0.013	0.073	0.006	0.002	0.011	0.037	0.011	0.073
V_R	0.097	0.090	0.104	0.634	0.589	0.709	0.096	0.089	0.103	0.648	0.586	0.705