Received: 8 July 2020 | Revised: 9 December 2020 | Accepted: 14 December 2020

DOI: 10.1111/mec.15804

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

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

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]

[1]Faculty of Biological Sciences, School of Biology, University of Leeds, Leeds, UK

[2]School of Biological Sciences, University of East Anglia, Norwich, UK

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

[4]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK

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

Correspondence
Alexandra M. Sparks, Faculty of Biological Sciences, School of Biology, University of Leeds, Leeds, UK.
Email: A.M.Sparks@leeds.ac.uk

Funding information
This work was supported by NERC grant (NE/B504106/1 to T.A.B. and D.S.R.), NWO Rubicon (825.09.013) and NERC (NE/I021748/1) fellowships to H.L.D., Lucie Burgers Foundation grant 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.).

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. Heritability of telomere length has primarily been calculated using parent–offspring regression which can be confounded by shared environments. To control for confounding variables, quantitative genetic "animal models" can be used, but few studies have applied animal models in wild populations. Furthermore, parental age at conception may also influence offspring telomere length, but most studies have been cross-sectional. We investigated within- and between-parental age at conception effects and heritability of telomere length in the Seychelles warbler using measures from birds caught over 20 years and a multigenerational 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). Animal models provided evidence that heritability and evolvability of telomere length were low in this population, and that variation in telomere length was not driven by early-life effects of hatch period or parental identities. Quantitative polymerase chain reaction plate had a large influence on telomere length variation and not accounting for it in the models would have underestimated heritability. Our study illustrates the need to include and account for technical variation in order to accurately estimate heritability, as well as other environmental effects, on telomere length in natural populations.

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

David S. Richardson and Hannah L. Dugdale authors are joint senior authors.

1 | INTRODUCTION

A complete understanding of the relative impact of genetic and environmental effects on senescence requires quantifying individual variation in senescence, but this is difficult to achieve, for instance because it can be difficult to quantify the costs of different conditions experienced, especially in wild populations (Nussey et al., 2008; de Pol & Verhulst, 2006; van 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), can shed light on the candidate mechanisms underlying senescence (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 (Campisi, 2005; Harley et al., 1992) which may lead to organismal senescence (López-Otín et al., 2013). However, telomeres can also be extended by telomerase (Greider & Blackburn, 1989) and alternative lengthening (Cesare & Reddel, 2010). Telomere shortening occurs with age in a wide range of species (e.g., Aubert et al., 2012; Salomons et al., 2009). Furthermore, whether causal, or just correlational (Simons, 2015; Young, 2018), telomere length relative to age positively predicts health (Blackburn et al., 2015; Boonekamp et al., 2013) 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 (Bauch et al., 2013; Bebbington et al., 2016; Bize et al., 2009; 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 & Richardson, 2018). Initial telomere length is inherited from each parent (Delgado et al., 2019). As a mother's gametes are produced prenatally, there could be selection for higher quality oocytes with longer telomeres to be used first (Monaghan et al., 2020). In contrast, fathers produce sperm throughout their life, and there is cross-sectional evidence from humans that sperm telomere length is positively correlated with age and older fathers have offspring with longer telomeres (Aston et al., 2012; Broer et al., 2013; Eisenberg et al., 2012; Kimura et al., 2008; Unryn et al., 2005). Such effects may be due to the activity of telomerase in the testes resulting in elongated telomeres with age (Aviv & Susser, 2013; Kimura et al., 2008). A second (not mutually exclusive) hypothesis is the selective survival or proliferation of germ stem cells with longer telomeres (Hjelmborg et al., 2015; Kimura et al., 2008). Alternatively, the positive association between paternal age and offspring telomere length may arise through selective disappearance, whereby males with short telomeres are lost from the population (Bauch et al., 2019; Noguera et al., 2018). Studies in nonhuman vertebrates report conflicting results; while some have found a positive correlation between offspring telomere length and paternal (Eisenberg et al., 2017) or maternal age (Asghar et al., 2015), others have found negative paternal age correlations (Bauch et al., 2019; Bouwhuis et al., 2018; Criscuolo et al., 2017; de Frutos et al., 2016; Noguera et al., 2018; Olsson et al., 2011) or no parental age effects (Belmaker et al., 2019; Froy et al., 2017; Heidinger et al., 2016; McLennan et al., 2018; van Lieshout, Sparks, et al., 2020). Importantly, only two of these studies have looked at longitudinal changes in offspring telomere length within mothers and fathers as they aged (Bauch et al., 2019; van Lieshout, Sparks, et al., 2020). This is important because the selective disappearance of parents with shorter telomeres may obscure age-related patterns (Bauch et al., 2019; Noguera et al., 2018). Work on a wider range of species, separating within- from between-parental age at conception effects, is required to identify the drivers of variation in parental age at conception effects.

In addition to parental age effects, genetic variation also influences the maintenance of telomeres from the first mitotic division, and thus telomere dynamics across an individual's lifetime (Delgado et al., 2019; Eisenberg, 2019). Environmental effects and life-history events are also associated with telomere shortening due to the stress they exert on organisms, and such effects will accumulate with age (Hall et al., 2004; Heidinger et al., 2012). The majority of the pioneering studies quantifying the contribution of genetic variation to telomere length have been in human populations and typically implicate significant heritability (Dugdale & Richardson, 2018). It is, however, difficult to interpret heritability estimates from human studies where processes, such as industrialization and medical interventions, limit their evolutionary interpretation. Importantly, biologists wanting to understand the ecological and evolutionary significance of telomere dynamics will be interested in studying telomere heritability in wild populations, experiencing natural environmental variation and where natural selection is occurring. To date, very few studies have attempted to separate genetic from environmental contributions to variation in telomere length within wild populations. Consequently, our understanding of the heritability of telomeres in wild populations is limited (Dugdale & Richardson, 2018). This is important as the amount of additive genetic variance underlying a trait, such as telomere length, limits the variation that selection can act on and, therefore, a trait's evolutionary potential (Lynch & Walsh, 1998).

In wild populations, telomere length heritability estimates range from 0 to 1 (Dugdale & Richardson, 2018). Heritability estimates have, however, been based primarily on parent–offspring regressions, which assume that the similarity between parents and offspring is genetic, when, in fact, relatives often also share environments. These shared environmental effects, including cohort and maternal effects (Asghar et al., 2015; Becker et al., 2015), will artificially inflate heritability estimates (Kruuk & Hadfield, 2007; Kruuk et al., 2008). Additionally, telomere length will change throughout life, so measures across the lifetimes of individuals will be the product of inherited telomere length, attrition and restoration/lengthening (Dugdale & Richardson, 2018). Not all telomere studies have taken individual age at sampling into account (Dugdale & Richardson, 2018), or sampled both offspring and parents at the same age, as

both sampling and accurate ageing are difficult in wild populations (but see Becker et al., 2015 and Asghar et al., 2015). Subsequently, it is unclear whether the variation in heritability estimates of telomere length in wild populations reflects biological 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 utilize the relationships in a pedigree to estimate additive genetic variance, thus maximizing 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, the power to estimate heritability will depend on the number of individuals phenotyped, pedigree size, pedigree structure (e.g., depth, completeness and family sizes), the confidence with which pedigree relationships were estimated (either using molecular data or observational data), as well as the complexity of the animal model (Wilson et al., 2010). For wild populations, these conditions are often less than optimal compared to animal breeding studies, with large sample sizes of phenotyped individuals often difficult to obtain, and with molecular pedigree relationships unlikely to be perfect (Wilson et al., 2010). Furthermore, statistical power will increase with the heritability in the population of interest (Morrissey & Wilson, 2010). While heritability estimates vary greatly in wild populations (Dugdale & Richardson, 2018), calculation of the repeatability – the amount of variance explained by individual identity in a data set with multiple measures – will provide the upper limit to ordinary heritability (Falconer & Mackay, 1996). Using this estimate, and given a data set and pedigree structure, simulation studies can predict the power to detect the expected range of heritabilities (Morrissey & Wilson, 2010). In humans, 10 studies using variance partitioning approaches have detected considerable genetic variation underlying telomere length, with heritabilities ranging from 0.07 to 0.70 (Blackburn, Charlesworth, et al., 2015; Broer et al., 2013; Coutts et al., 2019; Delgado et al., 2018; Faul et al., 2016; Honig et al., 2015; Kim et al., 2020; Lee et al., 2014; Njajou et al., 2007; Zhu et al., 2013). Currently, the six studies using animal model approaches in wild or captive animal populations have found heritabilities ranging from 0 to 1 (Asghar et al., 2015; Becker et al., 2015; Atema et al., 2015; van Lieshout, Sparks, et al., 2020; Boonekamp et al. 2020; Foley et al., 2020). However, power analyses were only provided for one study (van Lieshout, Sparks, et al., 2020) and only a few of these studies were able to separate out (some) common environment effects from additive genetic effects (Asghar et al., 2015; Becker et al., 2015; van Lieshout, Sparks, et al., 2020). In addition to calculating heritability, evolvability (a mean standardized measure of additive genetic variance) facilitates comparison between studies of the evolutionary potential of a given trait (Houle, 1992). There is a need for more studies assessing the heritability and evolvability of telomere length using large data sets with multigenerational pedigrees and animal model approaches.

In this study, we used long-term individual-based multigenerational 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 length decline is greatest in early life in the Seychelles warbler, and telomere length shows strong cohort effects and is positively associated with island-wide 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), social conflict (Bebbington et al., 2017) and parental care (Hammers et al., 2019). In the Seychelles warbler, telomere length is therefore an important biomarker of condition and senescence and is impacted by environmental conditions. The individual repeatability of relative telomere length (RTL) is 0.068 in this population (Spurgin et al., 2018), which sets a low upper limit on ordinary heritability (Falconer & Mackay, 1996). However, the relative contribution of other environmental components, such as parental, catch period and hatch period effects, is unknown.

Here, we estimate the heritability of telomere length in the Seychelles warbler using 2,663 telomere measures from 1,317 birds within a 10-generation genetic pedigree. First, we test for effects of parental age at conception on offspring telomere length accounting for the age at which offspring were sampled. We then estimate the heritability and evolvability of telomere length using animal models where we control for expected confounding effects. We predict estimates of heritability will be 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 understanding the evolutionary potential of telomeres in this population.

2 | MATERIALS AND METHODS

2.1 | Study system

The Seychelles warbler is a small passerine endemic to the Seychelles archipelago (Komdeur et al., 1991). The entire population (~320 adult individuals in 115 territories) on Cousin island (29 ha; 04°20′S, 55°40′E) has been monitored intensively since 1985 (Hammers et al., 2019; Komdeur, 1992; Raj Pant et al., 2019; Richardson et al., 2007). Seychelles warblers defend year-round territories in which a dominant male and female breed and most clutches contain just one egg (Komdeur, 1994; Richardson et al., 2001). The major breeding season runs from June to September, although some pairs also breed in the minor breeding season between January and March (Komdeur et al., 1991; Komdeur & 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 & Daan, 2005), which is positively associated with telomere length (Spurgin et al., 2018). In addition, this study benefits from being able to separate 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 are 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 a unique colour ring combination (Richardson et al., 2001). Age of unringed birds was estimated using lay, hatch or fledge dates, or alternatively if these dates were unknown, we used eye colour to determine whether the individual was in the first year of its life or not (Komdeur, 1991). Since 1990, blood samples (~25 μl) have been taken and stored at room temperature in absolute ethanol, thus allowing molecular sexing, parentage assignment (Hadfield et al., 2006; Richardson et al., 2001), 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 =19 years). Furthermore, the population is intensively monitored with high annual resighting rates (~0.92 ± 0.02 for birds ≤2 years and 0.98 ± 0.01 for older birds, Brouwer et al., 2010), so accurate hatch and death years are known (Hammers et al., 2015). Each fieldwork period (a minor and major breeding season each year) is assigned a unique identifier (for each unique combination of year and season) and catch records are assigned to the fieldwork period ID in which they occurred (henceforth "catch period ID") or hatch records to the closest fieldwork period ID (henceforth "hatch period ID").

2.2 | Telomere data

We used the telomere data set generated in Spurgin et al. (2018), which included birds caught and blood sampled between 1995 and 2014, when the data were most complete. RTL was estimated using qPCR (quantitative polymerase chain reaction; Barrett et al., 2013; Bebbington et al., 2016; Spurgin et al., 2018). DNA integrity (agarose gel) and 260/280 ratios were checked in all samples before running any qPCR, and any samples with signs of degradation were removed, reextracted and checked again. Within-plate repeatability was 0.74 (95% confidence intervals [CI] =0.74–0.75) and 0.73 (95% CI =0.71–0.74) for the GAPDH and telomere cq values, respectively (Spurgin et al., 2018). Between-plate repeatability of RTL, based on 422 samples measured at least twice at different time points (the different time points being a key point to replicate the actual technical error across normal samples), was 0.68 (95% CI =0.65–0.70) (Spurgin et al., 2018). All repeatability estimates were calculated on RTL measurements from the same DNA extraction and may be lower if DNA extractions were also repeated. There were no storage time effects (of the blood samples) on telomere length (Spurgin et al., 2018). Our cleaned data set included 2,664 samples from 1,318 individuals that passed quality control (Bebbington et al., 2016) and filtering steps (sample removal criteria: telomere cq ≥25 or cq replicate difference ≥0.5; GADPH cq ≤21 or ≥26 or cq replicate difference ≥0.5; RTL values ≥3). 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.

2.3 | 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 Supporting Information (Supplementary parentage methods, Figures S1–S3 and Tables S1–S3). Parentage was assigned at $p \geq .8$. The pruned pedigree, calculated using *pedantics* 1.7 (Morrissey & Wilson, 2010), included parentage assignments for individuals born in 1992–2014 and contained 1,482 informative individuals for telomere length with 1,217 maternities and 1,268 paternities (Table S3).

2.4 | Statistical analyses

2.4.1 | 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 (from checking model diagnostics) and to be consistent with previous work on this system (Spurgin et al., 2018), and in each model subset RTL was subsequently z-transformed (mean centred and divided by 1 *SD*) for comparability of telomere studies (Verhulst, 2020). Distributions of nontransformed and the square-root and z-transformed RTL data are provided in Figure S4 and conclusions were the same when nontransformed RTL was used in these models (see Tables S4–S5). Collinearity between the fixed effects was checked by calculating variance inflation factors (VIFs); 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 models following Spurgin et al., 2018), parental age at conception (maternal and paternal) and technician identity (factor: two levels) as our fixed effects. Random effects included offspring identity, maternal identity, paternal identity, catch period ID and qPCR plate.

Based on our data set and model structure we had ≥80% statistical power to detect paternal age at conception effect sizes of ≥0.02 (Figure S5) using a simulation-based power analysis in the package *simr* 1.0.5 (Green & MacLeod, 2016). This was equivalent to a correlation coefficient of .059 ($r=\beta^*[SD_x/SD_y]$ 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; Eisenberg et al., 2012, 2017; Nordfjäll et al., 2010). There was considerable variation in maternal and paternal ages at conception (Figure S6a,b) and a significant but weak correlation between the two (r = .12, t_{1154} = 4.08, p < .001, Figure S6c) 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 = 1,137 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 nestling 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 centring (van de Pol & Wright, 2009). In 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 & Wright, 2009). We found within-PAC and between-MAC effects but effect sizes were small (see Results). To aid interpretability and comparability between studies, we then calculated the correlation coefficient of these effects using the equation $r = \beta^*(SD_x/SD_y)$ where r is the correlation coefficient, β is the regression slope and SD is the standard deviation for x (within PAC or between MAC) and y (RTL). Our correlation coefficients using this method were very similar to those estimated using the formula $t/\mathrm{sqrt}(t^2 + df)$ using $df = n - 1 - k$ (since error degrees of freedom are difficult to ascertain in linear mixed models) from Nakagawa & Cuthill, 2007 (r = −.058 for the within-PAC effect and .056 for the between-MAC effect).

2.4.2 | Heritability of telomere length

We investigated the 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 ≥0.17 (Figure S7), determined in *pedantics* 1.7 (Morrissey & Wilson, 2010). These univariate models were fitted with nontransformed RTL as the response variable to estimate variance components on the scale the trait was measured (de Villemereuil et al., 2016). We used a z-transformation in the parental age at conception models to improve comparability of the effect sizes of fixed effects between qPCR studies (Verhulst, 2020). Because we are interested in the partitioning of variance components rather than investigating fixed effects in these animal models, z-transformation was not performed. Animal models were fitted with an increasingly complex fixed and random effect structure. This allowed us to test for confounds between random effects, and to investigate how the inclusion of random effects affected our estimates of heritability.

Model 1 included only individual identity to account for repeated measures (to calculate between-individual variation or "repeatability", n = 1,317 individuals). In model 2, individual identity was partitioned into additive genetic and permanent environment components using the pruned pedigree. In model 3, we included fixed effects of sex (factor), age (log-transformed following Spurgin et al., 2018) and technician (factor: two levels) to investigate how heritability was impacted by the inclusion of fixed effects (following: de Villemereuil et al., 2018; Wilson, 2008). In model 4, we estimated technical variance by adding qPCR plate ID as a random effect (271 levels). We subsequently added maternal (model 5; 380 mothers) and paternal (model 6; 354 fathers) identity, determined from the genetic pedigree, to investigate parental effects underlying telomere length. Maternal effects have previously been observed in other species (Asghar et al., 2015), and maternal inbreeding effects, but not paternal inbreeding effects, on offspring telomere length have been documented in our population (Bebbington et al., 2016). We then added the random effects of catch period ID (model 7; 37 levels) and current territory (model 8; 166 levels), to account for spatiotemporal factors associated with telomere length (Spurgin et al., 2018). Finally, we tested for early-life effects of hatch period ID (model 9; 39 levels) to account for long-lasting effects of natal conditions on telomere variation. Although we had information on natal territory, models including natal territory did not converge, and simpler models suggested that natal territory explained no variance in RTL.

We used default priors for fixed effects, while for the random effects (except for the residual variance structure which were inverse-Wishart priors, where V = 1, n = 0.002) we applied parameter expanded priors (with V = 1, nu =1, alpha.mu =0 and alpha.V = 1,000) as the variance estimates were close to zero (Hadfield, 2018). 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 =1,000; Models 6–9: 3.6×10^6 iterations, burn-in =6×10^5, thinning =1,500). To assess convergence of *MCMCglmm* models, we checked: autocorrelation r < .1, effective sample sizes ≥1,000, Heidelberger and Welch's tests were passed and Geweke tests were passed. For estimates of the fixed effects

TABLE 1 Linear mixed model results investigating between vs. within maternal and paternal age at conception (MAC and PAC, respectively) effects on offspring telomere length in the Seychelles warbler using the within-subject centring method (van de Pol & Wright, 2009)

	Model 1				Model 2			
Variables	**Estimate**	***SE***	**LRT**	***p***	**Estimate**	***SE***	**LRT**	***p***
Fixed effects								
Intercept	−0.302	0.092			−0.302	0.092		
Log age (years)	**−0.311**	**0.030**	**102.250**	**<.001**	**−0.311**	**0.030**	**102.250**	**<.001**
Sex (male)	0.024	0.039	0.374	.541	0.024	0.039	0.374	.541
Technician	**0.465**	**0.076**	**35.954**	**<.001**	**0.465**	**0.076**	**35.954**	**<.001**
MeanMAC	**0.030**	**0.011**	**7.482**	**.006**	**0.039**	**0.016**	**6.002**	**.014**
DevMeanMAC	−0.010	0.011	0.726	.394				
MAC					−0.010	0.011	0.726	.394
MeanPAC	0.001	0.009	0.011	.918	**0.033**	**0.015**	**5.102**	**.024**
DevMeanPAC	**−0.032**	**0.011**	**8.032**	**.005**				
PAC					**−0.032**	**0.011**	**8.032**	**.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			
Catch period ID	0.030				0.030			
Residual	0.653				0.653			

Associations were investigated in offspring telomere length of all ages (2,361 RTL measures of 1,156 offspring) and included are the estimated effects (estimate), standard errors (*SE*s), 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* values <.05 are shown in bold.

and random effects we took the mode of the posterior distributions. We defined fixed effects as significant if the 95% credible intervals (CrI) 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. In addition, we also calculated evolvability (a mean standardized measure of additive genetic variance) of telomere length by dividing the additive genetic variance by the squared trait's mean ($I_A = V_A/\bar{x}^2$ where I_A is evolvability, V_A is the additive genetic variance and $\bar{x}$ is the trait mean) (Houle, 1992).

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. The 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 cobreeding (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 $=4 \times 10^6$, thinning =3,000) using model 7's structure, as other random effects in models 8 and 9 explained a small proportion of the phenotypic variance (see Results).

3 | RESULTS

Maternal and paternal age at conception (MAC and PAC, respectively) were not significantly associated with offspring RTL when using telomere lengths across all ages, or when the data set was restricted to the first offspring measurements taken as nestlings, or when all measurements were taken from juvenile offspring (<1 year old; Figure S8, Table S6). However, when parental age at conception effects were separated into within- vs. between-parental age effects for lifelong RTL, there was a significant negative within-paternal age effect and a significant 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- vs. 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 (r = −.059) and between-maternal (r = .060) age effects on offspring RTL were small when converted to a correlation coefficient (Figure 1). There was no difference in lifelong RTL between the sexes, but there was a logarithmic association with age and an effect of technician (Table 1; Table S6).

We estimated heritability with a quantitative genetic animal model using a hierarchical approach (Figure 2). 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 S7: Model 1). As repeatability sets the upper limit on ordinary heritability (when indirect genetic effects are not considered), heritability estimates were also low across all models. RTL heritability was 0.080 (95% CrI: 0.041–0.144; Table S7: Model 2) in the simplest model and 0.031 (95% CrI: <0.001–0.067) after the inclusion of all fixed and random effects in the final model (Figure 2, Table 2: Model 9). Evolvability of RTL was low at 0.005 (95% CrI: <0.001–0.012) in the final model (Model 9). We found a small catch period effect and moderate qPCR plate effects in the final model (Table 2). There was no evidence for permanent environment, maternal or paternal, current territory or hatch period effects (Table 2). If plate variance was not included in the total phenotypic variance, as 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 catch period 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 S8). Without plate included in the total phenotypic variance, 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).

4 | DISCUSSION

We found a negative within-paternal age at conception effect, and a positive between-maternal age at conception effect on offspring telomere length in the Seychelles warbler, but both of these effect sizes were small. These results add to the growing literature reporting mixed results in wild populations (Asghar et al., 2015; Belmaker et al., 2019; Eisenberg, 2019) and contribute to the very few studies which have investigated parental age at conception effects longitudinally (Bauch et al., 2019; van Lieshout, Sparks, et al., 2020).

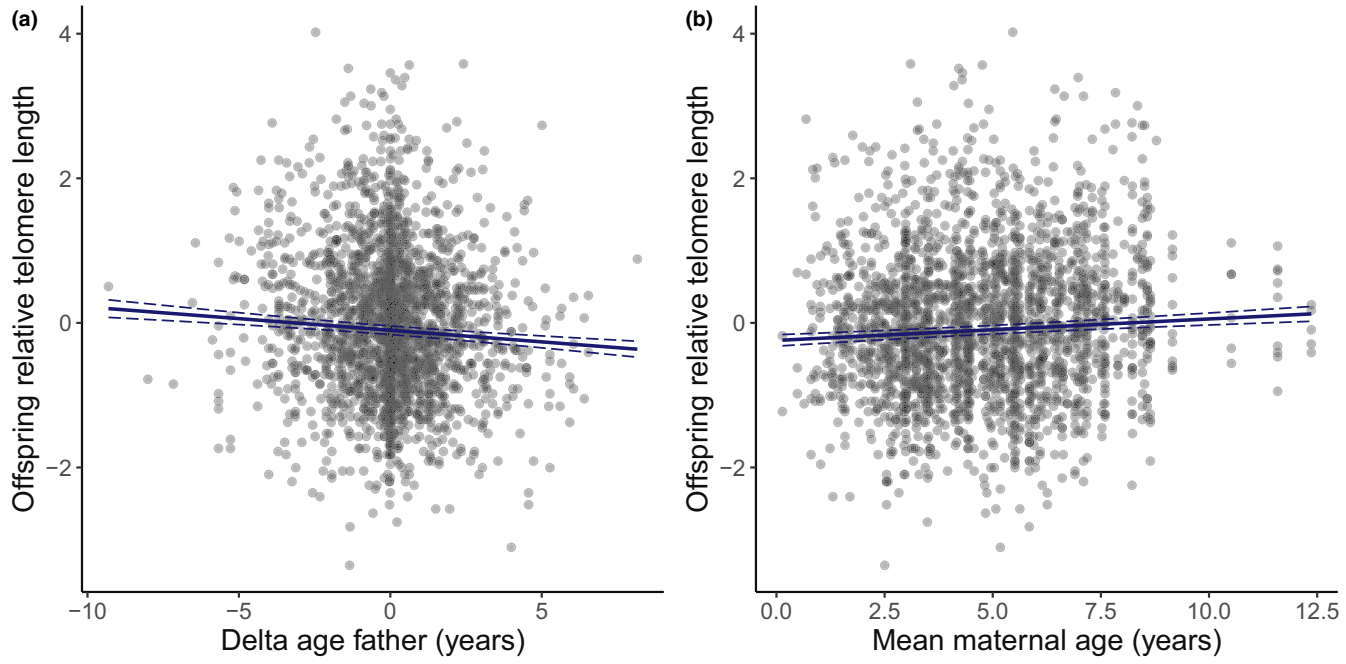

FIGURE 1 Scatterplots of relative telomere length (RTL) data (square-root and z-transformed) from the Seychelles warbler showing significant negative within-paternal age at conception effects (a) and positive between-maternal age at conception effects (b) on offspring telomere length across all ages (2,361 RTL measures of 1,156 offspring). Lines indicate linear mixed model predictions (Model 1, Table 1) using a within-subject centring method estimated for birds with average values for all other continuous fixed effects in the model with dashed lines indicating standard errors. Data points are semitransparent to show overlapping values

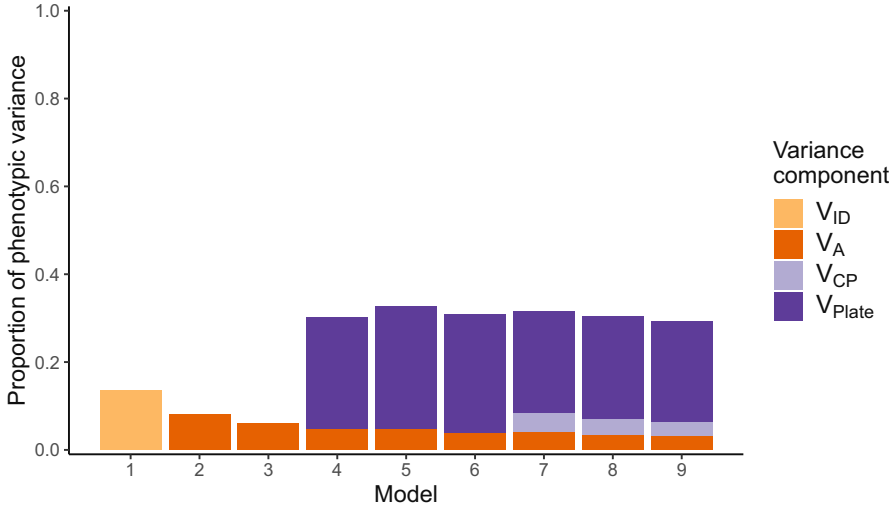

FIGURE 2 Estimated variance components as proportions of total phenotypic variance in relative telomere length determined using univariate models in the Seychelles warbler. Models were fitted additively with increasing random or fixed effects as follows: Model 1 – individual identity (V_{ID}), 2 – partitioning of V_{ID} into additive genetic (V_A) and permanent environment (V_{PE}) components, 3 – the addition of fixed effects (age, sex, technician), 4 – qPCR plate ID (V_{Plate}), 5 – maternal identity (V_{Mat}), 6 – paternal identity (V_{Pat}), 7 – catch period ID (V_{CP}), 8 – current territory (V_{Terr}) and 9 – hatch period ID (V_{HP}). Five variance components (V_{PE}, V_{Mat}, V_{Pat}, V_{Terr}, V_{HP}) explained <0.001 of the total phenotypic variance in all models so do not show up on the graph. For full model results see Table 2 and Table S7

TABLE 2 Animal model variance component estimates and their associated proportions of the total phenotypic variance from a Markov chain Monte Carlo model of relative telomere length in the Seychelles warbler

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_{CP}	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_{HP}	<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**			

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}), catch period ID (V_{CP}), current territory (V_{CTerr}), hatch period ID (V_{HP}), and residual (V_R) variance. Included are the variance component estimates as the posterior modes along with their lower and upper 95% credible intervals (CrI) and the proportion of the total phenotypic variance explained by the terms (Prop V_P) with their associated 95% CrIs. The significance of fixed effects was determined when the 95% CrIs did not overlap zero (shown in bold).

Animal models indicated a low heritability of telomere length, small catch period effects and moderate technical effects in the form of between-qPCR plate effects.

A number of human studies have documented a positive cross-sectional association between paternal age at conception and offspring telomere length (Broer et al., 2013; Eisenberg et al., 2012;

TABLE 3 Animal model variance component estimates and their associated proportions from a Markov chain Monte Carlo 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)

	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_{CP}	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

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}), catch period ID (V_{CP}), 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.

Unryn et al., 2005). 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 size of the correlation coefficients previously published (De Meyer et al., 2007; Eisenberg et al., 2012, 2017; Nordfjäll et al., 2010). However, using within-subject centring we found weak but significant within-paternal age at conception and between-maternal age at conception effects on offspring telomere length. In contrast to cross-sectional findings in humans (Broer et al., 2013; Eisenberg et al., 2012; Unryn et al., 2005), we found that males produced offspring with shorter telomere lengths as they aged. Furthermore, we also found that females that lived longer had offspring with longer telomeres. However, both these effects on offspring telomere length were relatively small when considered as correlation coefficients.

Despite the consistency in human studies, studies in nonhuman 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 (Dupont et al., 2018; Eisenberg et al., 2017), most find a negative paternal age at conception effect (Bauch et al., 2019; Bouwhuis et al., 2018; Criscuolo et al., 2017; de Frutos et al., 2016; Noguera et al., 2018; Olsson et al., 2011). Furthermore, many studies have documented no parental age at conception effects (Belmaker et al., 2019; Froy et al., 2017; Heidinger et al., 2016; McLennan et al., 2018; van Lieshout, Sparks, et al., 2020), while one study found a positive maternal but no paternal age at conception effect (Asghar et al., 2015). Such mixed results in natural populations may be due to the majority of studies on parental age at conception effects being cross-sectional. Where telomere length is known to be positively associated with survival in a population (Wilbourn et al., 2018), this could lead to the selective disappearance of parents with shorter telomeres from older age groups. Such selective disappearance could obscure within-parental age at conception effects when analysing data at a cross-sectional level (Bauch et al., 2019; Noguera et al., 2018). So far, only two published studies investigating parental age at conception effects have separated within- from between-parental age effects: a negative paternal age at conception effect was found in wild jackdaws (Bauch et al., 2019) while no parental age at conception effects were found in wild European badgers (van Lieshout, Sparks, et al., 2020). More studies using a within-subject centring approach (van de Pol & Wright, 2009) are now needed in both human and natural populations to determine whether such heterogeneous findings are indeed driven by cross-sectional approaches.

We investigated heritability using an animal model approach. Telomere length had a low 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) and evolvability 0.005 (95% CrI: <0.001–0.012). The six studies in wild or captive animal populations that have previously estimated heritability using animal models found either no significant (Becker et al., 2015; van Lieshout, Sparks, et al., 2020), very low (0.011, 95% CI:

<0.001–0.042, to 0.060, 95% CI: 0.023–0.106 depending on prior specification, Foley et al., 2020) or – in contrast – large (0.48, 95% CI: 0.24–0.72, Asghar et al., 2015; 0.99, 95% CrI: 0.87–1, Atema et al., 2015) narrow-sense heritabilities of telomere length, and a large broad-sense heritability of 1.24 (95% CI: 0.83–1.65, Boonekamp et al. 2020). However, whether such differences represent true biological variation between species, or whether these differences may also reflect methodological differences is unclear. While qPCR is the most common method used in nonmodel vertebrate studies (because it is cheaper and of higher throughput), terminal restriction fragment (TRF) analyses or southern blot methods are more technically repeatable (Aviv et al., 2011). Despite the lower technical repeatability of the qPCR method compared to the TRF method, there is evidence of high correlations (r = .847–.896, p < .001) between these two measures (Aviv et al., 2011; Tarik et al., 2018). Higher individual repeatabilities (i.e., the proportion of phenotypic variance explained by individual identity), or within-individual correlations, have been documented in TRF studies (Bauch et al., 2013; Bichet et al., 2020; Boonekamp et al., 2014) than those in qPCR studies in wild populations (Fairlie et al., 2016; Foley et al., 2020; van Lieshout, Sparks, et al., 2020; Spurgin et al., 2018). The presence of telomere lengthening, which has been documented in qPCR studies including our study population (Fairlie et al., 2016; van Lieshout et al., 2019; Spurgin et al., 2018), may be an important factor contributing to a lower between-individual repeatability.

Of the six studies using animal models in nonmodel species, the two captive studies which found high heritabilities of ~1 used the TRF method (Atema et al., 2015; Boonekamp *et al*. 2020), while the remaining qPCR studies which were conducted in wild populations found heritabilities of 0–0.48 (Asghar et al., 2015; Becker et al., 2015; Foley et al., 2020; van Lieshout, Sparks, et al., 2020). In humans there is also some evidence that within-individual correlations in telomere length measures are higher with TRF studies than qPCR studies (Benetos et al., 2013; Martin-Ruiz et al., 2005; Svenson et al., 2011). Despite this, the mean heritability estimate in 10 studies of humans using both qPCR measures and a variance partitioning approach was moderate at 0.47 (range 0.07–0.70) (Blackburn, Charlesworth, et al., 2015; Broer et al., 2013; Coutts et al., 2019; Delgado et al., 2018; Faul et al., 2016; Honig et al., 2015; Kim et al., 2020; Lee et al., 2014; Njajou et al., 2007; Zhu et al., 2013). Furthermore, a qPCR study in dairy cattle found heritabilities of 0.32 and 0.38 in the calf and cow data set, respectively (Seeker et al., 2018). In a recent simulation study of qPCR telomere data, measurement error alone produced patterns of low correlations between consecutive telomere measures within an individual, although this study highlights that this does not necessarily mean that all low correlations are due to measurement error (Nettle et al., 2019). The presence of these differences highlights the need for studies that apply both qPCR and TRF methods to the same samples in order to understand how repeatabilities and heritabilities are influenced by each methodology.

The low heritability of telomere length in the Seychelles warbler is consistent with individuals with longer telomeres having a higher probability of surviving until the next year, independent of age (Barrett et al., 2013). Although it is unclear whether the links between telomere length and fitness are causal (Young, 2018), it is possible that selection for longer telomeres in this population has reduced the genetic variation, and hence the heritability, of this trait (Falconer & Mackay, 1996). Previously we found a weak but significant positive association between food availability and telomere length (see Figure 3c, Spurgin et al., 2018), suggesting a relationship between environmental variation and telomere length. However, we could not identify any considerable sources of environmental variation in telomere length in this population. After accounting for common environmental effects (parental, territory and period [both catch and hatch] effects) we found evidence for only small catch period effects (with plate variance: 0.032, 95% CrI: 0.013–0.079) with residual variance still explaining the majority of the variation in telomere length (with plate variance: 0.635, 95% CrI: 0.588–0.703). These results indicate that telomere measurements are highly dynamic, and are not driven by early life effects of hatch period or parental effects in this population, and indicate little potential for telomere length to respond to selection.

Previous studies which investigated telomere heritability in wild populations using an animal model approach, and estimated other common environment 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 hatch 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 the European badger *Meles meles*, there was no significant heritability of telomere length, but moderate year (0.321, 95% CrI: 0.155–0.483) and small cohort (0.035, 95% CrI: 0.007–0.079) effects were present (van Lieshout, Sparks, et al., 2020). 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. Furthermore, we found no evidence for early-life effects (hatch period or parental effects) on lifelong telomere length variation: our final model only provided evidence for small effects of catch period on telomere length variation (without plate variance: 0.036, 95% CrI: 0.018–0.101). This contrasts with a number of studies which have observed higher telomere declines 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 (Raj Pant et al., 2019; Richardson et al., 2001), 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, we previously 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 data set were measured as nestlings (12% chicks). Due to the inaccessibility of nests, which may be up to 30 m high in trees, individuals are usually caught as fledglings during their first 3 months when they remain dependent on their parents (Brouwer et al., 2006; Komdeur, 1994). While early-life measures of telomeres will be closer to the inherited telomere length, by using a measure of telomere length across the lifetimes of birds we are measuring a product of inheritance, attrition and restoration/lengthening. After birth, telomere attrition occurs rapidly (Hall et al., 2004; Salomons et al., 2009) and telomere length decreases with age quickest in the first few weeks of life in the Seychelles warbler (Spurgin et al., 2018). With more samples from younger or older individuals it would be possible to investigate how different genetic and environmental effects contribute to telomere length variation at different time points. Furthermore, we could have tested for genetic correlations between telomere measures in early and late life and investigated the presence of genotype-by-age interactions. However, our power to calculate heritabilities was lower using measures taken only as nestlings (N = 324 measures of 319 birds, power ≥0.80 to detect heritabilities of ≥0.23) or individuals showing senescent declines in reproduction and survival (>7 years Hammers et al., 2015; N = 249 measures of 161 birds, power ≥0.80 to detect heritabilities of ≥0.40). Future studies investigating how additive genetic variance in telomere length changes with age, investigating genetic correlations between early and late life telomere length, and whether telomere length is underpinned by any loci of major effect are warranted to understand the genetic architecture of telomere length (Dugdale & Richardson, 2018).

An important finding from our study is the impact of technical variation on telomere length measurements. Storage time did not affect telomere length (Spurgin et al., 2018). In contrast, the technician handling the qPCR did have an effect on RTL estimates, and we did find considerable plate effects. While the golden sample should standardize samples within a plate to minimize 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. Only one of the previous qPCR studies (van Lieshout, Sparks, et al., 2020) estimating heritability of telomere length using animal models included technical effects in their analyses, probably because other studies did not have enough levels to include these as random effects. 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 and method, changes in reagents, and extraction method effects (Eisenberg et al., 2015; Reichert et al., 2017; Seeker et al., 2016; van Lieshout, Froy, et al., 2020). Such systematic measurement error should be incorporated into analyses and reported to prevent it from biasing results (Nettle et al., 2019). Furthermore, other nonsystematic sources of error are present in qPCR studies due to the calculation of RTL from the ratio of telomeric DNA to a control gene. Measurement errors in both of these measures can magnify the error in the resulting ratio (Nettle et al., 2019).

While our technical repeatability was high (Spurgin et al., 2018) this was estimated based on RTL measurements from the same DNA extraction. Although we check for DNA integrity and quality prior to running the qPCR, it may be that our technical repeatability would be lower if we also repeated the DNA extraction. Importantly, while there was more error in our between-plate repeatability estimates, it must be noted that these were calculated based on plates run with a considerable time gap between the repeats to more accurately reflect technical error in our data set. In this study, while we have accounted for technician effects, tested for storage time effects and accounted for between-plate variation (both with the golden sample and also by including plate ID in models), we cannot rule out that the remaining large residual variance (although not uncommon in quantitative genetic studies Bérénos et al., 2014) may also be due to within-plate variation (Eisenberg et al., 2015). Within-plate variation can occur due to edge effects, from poor sealing and/or from thermal gradient distortion at plate edges (Rogers-Broadway & Karteris, 2015), and in one study these row effects explained 1.3% of telomere length variance (van Lieshout, Sparks, et al., 2020). As a result, our heritability estimates may actually represent a lower limit of true heritability in this population. We currently run, on the same plate, three technical replicates of each sample, from the same DNA extraction, of both the telomere and control gene sequences (Barrett et al., 2013), with samples randomly assigned to plates (Spurgin et al., 2018). In future, a better solution would be to repeat this process with a separate DNA extraction with sample identity and well location added as an extra variance component in analyses. Importantly, between-plate repeatability estimates should also be carried out at two different time points representing the maximum time difference between the first and last samples being run in the data set.

In conclusion, our results find a low lower limit on the heritability of telomere length in this wild avian population. In our population, telomere length variation across an individual's lifetime was not driven by environmental variation in early life (hatch period or parental effects). Instead, telomere length was largely driven by unexplained variation, caused either by unmeasured technical or by environmental variation. In addition, 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 on offspring telomere length. 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 facilitating work on Cousin Island. We thank all those who have contributed to long-term data collection in the Seychelles warbler project, Owen Howison for maintenance of the Seychelles warbler database Jarrod Hadfield for advice on *MasterBayes* and *MCMCMglmm* and two anonymous reviewers. 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.

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.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.vt4b8gtr1 (Sparks et al., 2021). R scripts for the analysis are available at https://github.com/Seychelle-Warbler-Project/Sparks_2021_MolEcol

ORCID

Alexandra M. Sparks https://orcid.org/0000-0002-7697-4632
Lewis G. Spurgin https://orcid.org/0000-0002-0874-9281
Terry Burke https://orcid.org/0000-0003-3848-1244
David S. Richardson https://orcid.org/0000-0001-7226-9074
Hannah L. Dugdale https://orcid.org/0000-0001-8769-0099

REFERENCES

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

Aston, K. I., Hunt, S. C., Susser, E., Kimura, M., Factor-Litvak, P., Carrell, D., & Aviv, A. (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.

Atema, E., Mulder, E., Dugdale, H. L., Briga, M., van Noordwijk, A. J., & Verhulst, S. (2015). Heritability of telomere length in the Zebra Finch. *Journal of Ornithology*, *156*, 1113–1123.

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

Aviv, A., Hunt, S. C., Lin, J., Cao, X., Kimura, M., & Blackburn, E. (2011). Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. *Nucleic Acids Research*, *39*, e134–e134.

Aviv, A., & Susser, E. (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., Burke, T. A., Hammers, M., Komdeur, J., & Richardson, D. S. (2013). Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology*, *22*, 249–259.

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

Bauch, C., Becker, P. H., & Verhulst, S. (2013). Telomere length reflects phenotypic quality and costs of reproduction in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20122540.

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

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

Becker, P. J. J., Reichert, S., Zahn, S., Hegelbach, J., Massemin, S., Keller, L. F., Postma, E., & Criscuolo, F. (2015). Mother-offspring and nestmate 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., Hallinger, K. K., Glynn, R. A., Winkler, D. W., & Haussmann, M. F. (2019). The environmental and genetic determinants of chick telomere length in Tree Swallows (*Tachycineta bicolor*). *Ecology and Evolution*, *9*, 8175–8186.

Benetos, A., Kark, J. D., Susser, E., Kimura, M., Sinnreich, R., Chen, W., Steenstrup, T., Christensen, K., Herbig, U., Von Bornemann Hjelmborg, J., Srinivasan, S. R., Berenson, G. S., Labat, C., & Aviv, A. (2013). Tracking and fixed ranking of leukocyte telomere length across the adult life course. *Aging Cell*, *12*, 615–621.

Bérénos, C., Ellis, P. A., Pilkington, J. G., & Pemberton, J. M. (2014). Estimating quantitative genetic parameters in wild populations: A comparison of pedigree and genomic approaches. *Molecular Ecology*, *23*, 3434–3451.

Bichet, C., Bouwhuis, S., Bauch, C., Verhulst, S., Becker, P. H., & Vedder, O. (2020). Telomere length is repeatable, shortens with age and reproductive success, and predicts remaining lifespan in a long-lived seabird. *Molecular Ecology*, *29*, 429–441.

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

Blackburn, E. H. (1991). Structure and function of telomeres. *Nature*, *350*, 569–573.

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

Blackburn, N. B., Charlesworth, J. C., Marthick, J. R., Tegg, E. M., Marsden, K. A., Srikanth, V., Blangero, J., Lowenthal, R. M., Foote, S. J., & Dickinson, J. L. (2015). A retrospective examination of mean relative telomere length in the Tasmanian Familial Hematological Malignancies Study. *Oncology Reports*, *33*, 25–32.

Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., & Verhulst, S. (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*, 20133287–20133287.

Boonekamp, J., Rodríguez-Muñoz, R., Hopwood, P., Zuidersma, E., Mulder, E., Wilson, A., Verhulst, S., & Tregenza, T. (2020). Telomere length is highly heritable and independent of growth rate manipulated by temperature in field crickets. *bioRxiv*, https://doi.org/10.1101/2020.05.29.123216

Boonekamp, J. J., Simons, M. J. P., Hemerik, L., & Verhulst, S. (2013). Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging Cell*, *12*, 330–332.

Bouwhuis, S., Verhulst, S., Bauch, C., & Vedder, O. (2018). Reduced telomere length in offspring of old fathers in a long-lived seabird. *Biology Letters*, *14*, 20180213.

Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G., Albrecht, E., Amin, N., Beekman, M., de Geus, E. J. C., Henders, A., Nelson, C. P., Steves, C. J., Wright, M. J., de Craen, A. J. M., Isaacs, A., Matthews, M., Moayyeri, A., Montgomery, G. W., … Boomsma, D. I. (2013). Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *European Journal of Human Genetics*, *21*, 1163–1168.

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

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

Butler, D. G., Cullis, B. R., Gilmour, A. R., & Gogel, B. J. (2009). *Mixed Models for S Language Environments: ASReml-R Reference Manual*. Queensland Department of Primary Industries and Fisheries, Brisbane.

Campisi, J. (2005). Senescent cells, tumor suppression, and organismal aging: Good citizens, bad neighbors. *Cell*, *120*, 513–522.

Cesare, A. J., & Reddel, R. R. (2010). Alternative lengthening of telomeres: Models, mechanisms and implications. *Nature Reviews Genetics*, *11*, 319–330.

Charmantier, A., Brommer, J. E., & Nussey, D. H. (2014). The quantitative genetics of senescence in wild animals. A. Charmantier, D. Garant & L. E. B. Kruuk (Eds.). *Quantitative Genetics in the Wild* (pp. 68–83). Oxford, UK: Oxford University Press.

Coutts, F., Palmos, A. B., Duarte, R. R. R., de Jong, S., Lewis, C. M., Dima, D., & Powell, T. R. (2019). The polygenic nature of telomere length and the anti-ageing properties of lithium. *Neuropsychopharmacology*, *44*, 757–765.

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

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

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

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

de Villemereuil, P., Schielzeth, H., Nakagawa, S., & Morrissey, M. (2016). General methods for evolutionary quantitative genetic inference from generalized mixed models. *Genetics*, *204*, 1281–1294.

Delgado, D. A., Zhang, C., Chen, L. S., Gao, J., Roy, S., Shinkle, J., Sabarinathan, M., Argos, M., Tong, L., Ahmed, A., Islam, T., Rakibuz-Zaman, M., Sarwar, G., Shahriar, H., Rahman, M., Yunus, M., Jasmine, F., Kibriya, M. G., Ahsan, H., & Pierce, B. L. (2018). Genome-wide association study of telomere length among South Asians identifies a second RTEL1 association signal. *Journal of Medical Genetics*, *55*, 64–71.

Delgado, D. A., Zhang, C., Gleason, K., Demanelis, K., Chen, L. S., Gao, J., Roy, S., Shinkle, J., Sabarinathan, M., Argos, M., Tong, L., Ahmed, A., Islam, T., Rakibuz-Zaman, M., Sarwar, G., Shahriar, H., Rahman, M., Yunus, M., Doherty, J. A., … Pierce, B. L. (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., & Richardson, D. S. (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., Barbraud, C., Chastel, O., Delord, K., Ruault, S., Weimerskirch, H., & Angelier, F. (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., Burke, T., & Dugdale, H. L. (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., Hayes, M. G., & Kuzawa, C. W. (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., Kuzawa, C. W., & Hayes, M. G. (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., Tackney, J., Cawthon, R. M., Cloutier, C. T., & Hawkes, K. (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., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L., & Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell*, *15*, 140–148.

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics* (4th ed.) Harlow, UK: Longman. ISBN:0582243025.

Faul, J. D., Mitchell, C. M., Smith, J. A., & Zhao, W. (2016). Estimating telomere length heritability in an unrelated sample of adults: Is heritability of telomere length modified by life course socioeconomic status? *Biodemography and Social Biology*, *62*, 73–86.

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

Froy, H., Bird, E. J., Wilbourn, R. V., Fairlie, J., Underwood, S. L., Salvo-Chirnside, E., Pilkington, J. G., Bérénos, C., Pemberton, J. M., & Nussey, D. H. (2017). No evidence for parental age effects on offspring leukocyte telomere length in free-living Soay sheep. *Scientific Reports*, *7*, 9991.

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

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

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

Hadfield, J. (2018). *MCMCglmm Course Notes*. Available from: https://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf

Hadfield, J. D., Richardson, D. S., & Burke, T. (2006). Towards unbiased parentage assignment: Combining genetic, behavioural and spatial data in a Bayesian framework. *Molecular Ecology*, *15*, 3715–3730.

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

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

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

Hammers, M., Richardson, D. S., Burke, T., & Komdeur, J. (2012). Age-dependent terminal declines in reproductive output in a wild bird. *PLoS One*, *7*, e40413.

Hammers, M., Richardson, D. S., Burke, T., & Komdeur, J. (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.

Harley, C. B., Vaziri, H., Counter, C. M., & Allsopp, R. C. (1992). The telomere hypothesis of cellular aging. *Experimental Gerontology*, *27*, 375–382.

Heidinger, B. J., Blount, J. D., Boner, W., Griffiths, K., Metcalfe, N. B., & Monaghan, P. (2012). Telomere length in early life predicts lifespan. *Proceedings of the National Academy of Sciences*, *109*, 1743–1748.

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

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

Honig, L. S., Kang, M. S., Cheng, R., Eckfeldt, J. H., Thyagarajan, B., Leiendecker-Foster, C., Province, M. A., Sanders, J. L., Perls, T., Christensen, K., Lee, J. H., Mayeux, R., & Schupf, N. (2015). Heritability of telomere length in a study of long-lived families. *Neurobiology of Aging*, *36*, 2785–2790.

Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics*, *130*, 195–204.

Kim, J.-H., Nam, C. M., Lee, D., Bang, H., Ko, J.-H., Lim, I., Kim, G. J., Koes, B. W., & Lee, D.-C. (2020). Heritability of telomere length across three generations of Korean families. *Pediatric Research*, *87*, 1060–1065.

Kimura, M., Cherkas, L. F., Kato, B. S., Demissie, S., Hjelmborg, J. B., Brimacombe, M., Cupples, A., Hunkin, J. L., Gardner, J. P., Lu, X., Cao, X., Sastrasinh, M., Province, M. A., Hunt, S. C., Christensen, K., Levy, D., Spector, T. D., & Aviv, A. (2008). Offspring's leukocyte telomere length, paternal age, and telomere elongation in sperm. *PLoS Genetics*, *4*, e37.

Komdeur, J. (1991). *Cooperative breeding in the Seychelles warbler*, PhD thesis. Cambridge, UK: University of Cambridge.

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). 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., Bullock, I. D., & Rands, M. R. W. (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., & Daan, S. (2005). Breeding in the monsoon: Semi-annual reproduction in the Seychelles warbler (*Acrocephalus sechellensis*). *Journal of Ornithology*, *146*, 305–313.

Komdeur, J., Piersma, T., Kraaijeveld, K., Kraaijeveld-Smit, F., & Richardson, D. S. (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., & Hadfield, J. D. (2007). How to separate genetic and environmental causes of similarity between relatives. *Journal of Evolutionary Biology*, *20*, 1890–1903.

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

Lee, J. H., Cheng, R., Honig, L. S., Feitosa, M., Kammerer, C. M., Kang, M. S., Schupf, N., Lin, S. J., Sanders, J. L., Bae, H., Druley, T., Perls, T., Christensen, K., Province, M., & Mayeux, R. (2014). Genome wide association and linkage analyses identified three loci—4q25, 17q23.2, and 10q11.21—associated with variation in leukocyte telomere length: The Long Life Family Study. *Frontiers in Genetics*, *4*, 310.

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

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

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*, Sunderland, MA: Sinauer Associates.

Martin-Ruiz, C. M., Gussekloo, J., van Heemst, D., von Zglinicki, T., & Westendorp, R. G. J. (2005). Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: A population-based study. *Aging Cell*, *4*, 287–290.

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

Monaghan, P., Maklakov, A. A., & Metcalfe, N. B. (2020). Intergenerational transfer of ageing: Parental age and offspring lifespan. *Trends in Ecology & Evolution*, *35*, 927–937.

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

Nakagawa, S., & Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biological Reviews*, *82*, 591–605.

Nakagawa, S., Gemmell, N. J., & Burke, T. (2004). Measuring vertebrate telomeres: Applications and limitations. *Molecular Ecology*, *13*, 2523–2533.

Nettle, D., Seeker, L., Nussey, D., Froy, H., & Bateson, M. (2019). Consequences of measurement error in qPCR telomere data: A simulation study. *PLoS One*, *14*, e0216118.

Njajou, O. T., Cawthon, R. M., Damcott, C. M., Wu, S. H., Ott, S., Garant, M. J., Blackburn, E. H., Mitchell, B. D., Shuldiner, A. R., & Hsueh, W. C. (2007). Telomere length is paternally inherited and is associated with parental lifespan. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 12135–12139.

Noguera, J. C., Metcalfe, N. B., & Monaghan, P. (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., Svenson, U., Norrback, K.-F., Adolfsson, R., & Roos, G. (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., Coulson, T., Festa-Bianchet, M., & Gaillard, J.-M. (2008). Measuring senescence in wild animal populations: Towards a longitudinal approach. *Functional Ecology*, *22*, 393–406.

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

Ponzi, E., Keller, L. F., Bonnet, T., & Muff, S. (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*, Vienna, Austria: R Foundation for Statistical Computing.

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

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

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

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

Rogers-Broadway, K. R., & Karteris, E. (2015). Amplification efficiency and thermal stability of qPCR instrumentation: Current landscape and future perspectives. *Experimental and Therapeutic Medicine*, *10*, 1261–1264.

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

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

Seeker, L. A., Ilska, J. J., Psifidi, A., Wilbourn, R. V., Underwood, S. L., Fairlie, J., Holland, R., Froy, H., Salvo-Chirnside, E., Bagnall, A., Whitelaw, B., Coffey, M. P., Nussey, D. H., & Banos, G. (2018). Bovine telomere dynamics and the association between telomere length and productive lifespan. *Scientific Reports*, *8*, 12748.

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

Sparks, A., Spurgin, L., van der Velde, M., Fairfield, E., Komdeur, J., Burke, T., Richardson, D., & Dugdale, H. (2021). Data from: Telomere heritability and parental age at conception effects in a wild avian population. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.vt4b8gtr1

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

Svenson, U., Nordfjäll, K., Baird, D., Roger, L., Osterman, P., Hellenius, M.-L., & Roos, G. (2011). Blood cell telomere length is a dynamic feature (ed S Cotterill). *PLoS One*, *6*, e21485.

Tarik, M., Ramakrishnan, L., Sachdev, H. S., Tandon, N., Roy, A., Bhargava, S. K., & Pandey, R. M. (2018). Validation of quantitative polymerase chain reaction with Southern blot method for telomere length analysis. *Future Science OA*, *4*, FSO282.

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

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

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

van Lieshout, S. H. J., Bretman, A., Newman, C., Buesching, C. D., Macdonald, D. W., & Dugdale, H. L. (2019). Individual variation in early-life telomere length and survival in a wild mammal. *Molecular Ecology*, *28*, 4152–4165.

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

van Lieshout, S. H., Sparks, A. M., Bretman, A., Newman, C., Buesching, C. D., Burke, T., Macdonald, D. W., & Dugdale, H. L. (2020). Estimation of environmental, genetic and parental age at conception effects on telomere length in a wild mammal. *Journal of Evolutionary Biology*, *00*, 1–13.

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., Bolton, M., & Monaghan, P. (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., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., & Boonekamp, J. J. (2018). The relationship between telomere length and mortality risk in nonmodel 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., Réale, D., Clements, M. N., Morrissey, M. M., Postma, E., Walling, C. A., Kruuk, L. E. B., & Nussey, D. H. (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.

Zhu, Y., Voruganti, V. S., Lin, J., Matsuguchi, T., Blackburn, E., Best, L. G., Lee, E. T., MacCluer, J. W., Cole, S. A., & Zhao, J. (2013). QTL mapping of leukocyte telomere length in American Indians: The strong heart family study. *Aging*, *5*, 704–716.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Sparks AM, Spurgin LG, Velde M, et al. Telomere heritability and parental age at conception effects in a wild avian population. *Mol Ecol*. 2022;31:6324–6338. https://doi.org/10.1111/mec.15804