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

Running title: Heritability of telomere length

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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

≥0.17 (Figure S6), determined in *Pedantics* 1.7 (Morrissey and Wilson 2010). These univariate models were fitted with RTL (non-transformed) as the response variable and had 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'). 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: 2 levels) to investigate how heritability was impacted by the inclusion of fixed effects (following: Wilson 2008; de Villemereuil et al. 2018). In model 4 we estimated technical variance by adding qPCR plate ID as a random effect. We subsequently added maternal (model 5) and paternal (model 6) 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 season of capture (model 7) and current territory (model 8), to account for spatio-temporal factors associated with telomere length (Spurgin et al. 2018). Finally, we tested for early-life effects of birth season (model 9) 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=1000) as the variance estimates were close to zero (Hadfield 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.

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Data Accessibility

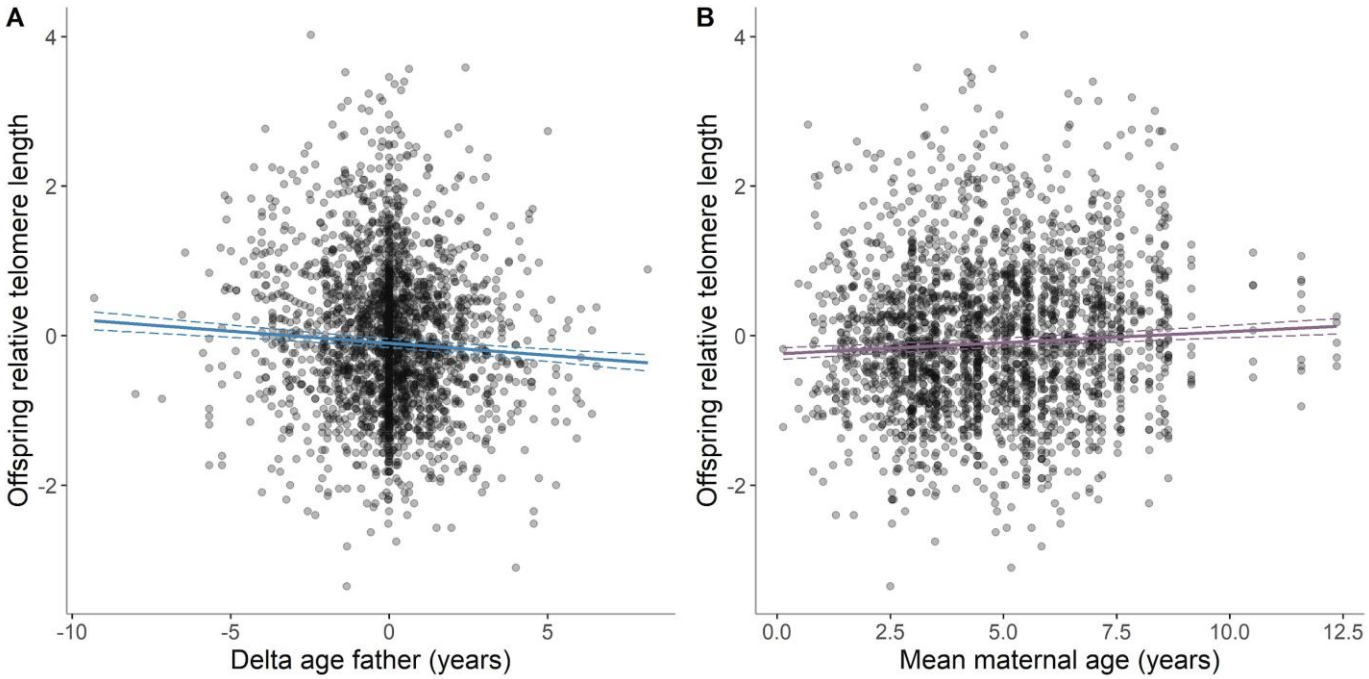
Data will be deposited in the Dryad Digital Repository upon acceptance.

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.

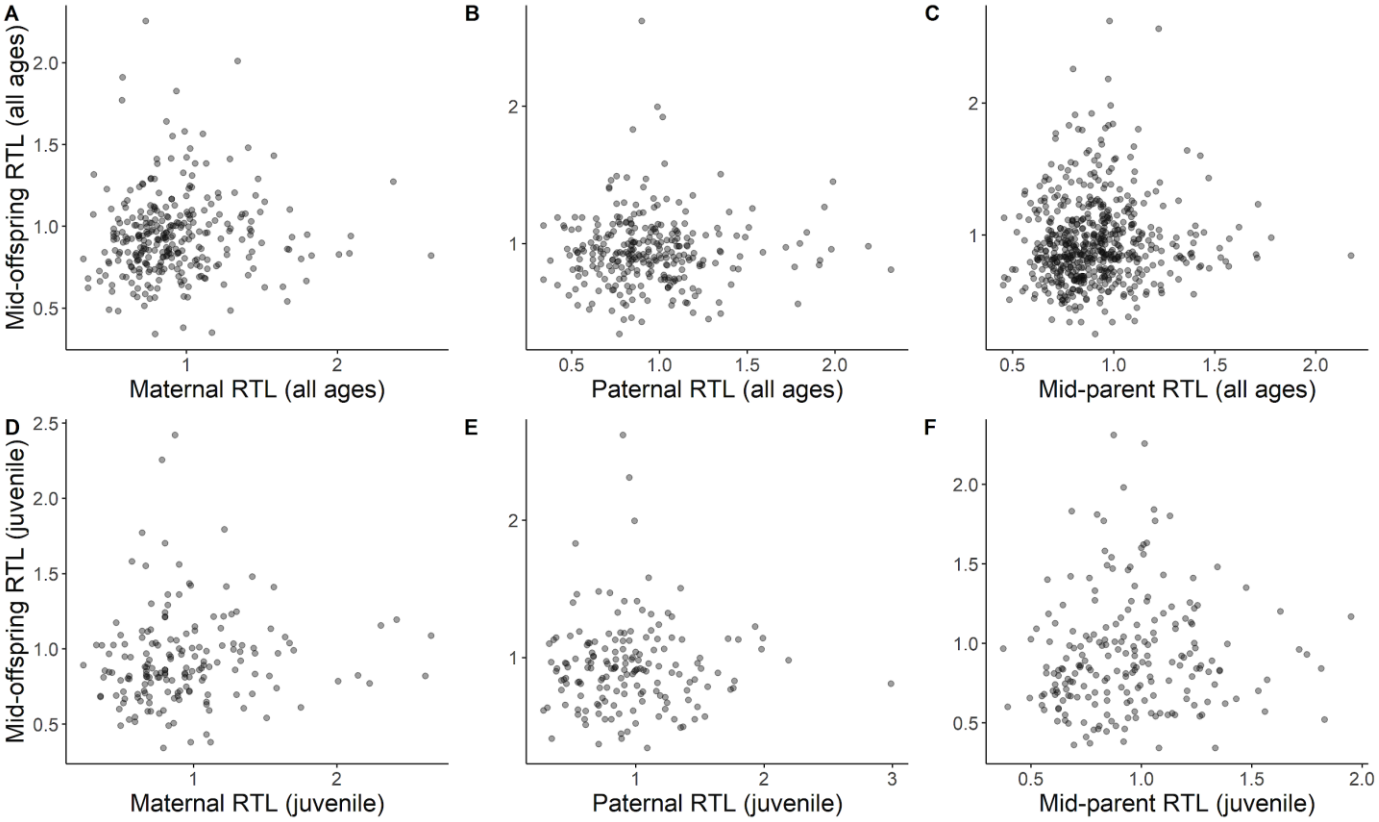
Table 1. Linear mixed model results investigating between versus within maternal and paternal age at conception (MAC and PAC, respectively) effects on offspring telomere length in the Seychelles warbler using the within-subject centering method (van de Pol and Wright 2009). Associations were investigated 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			



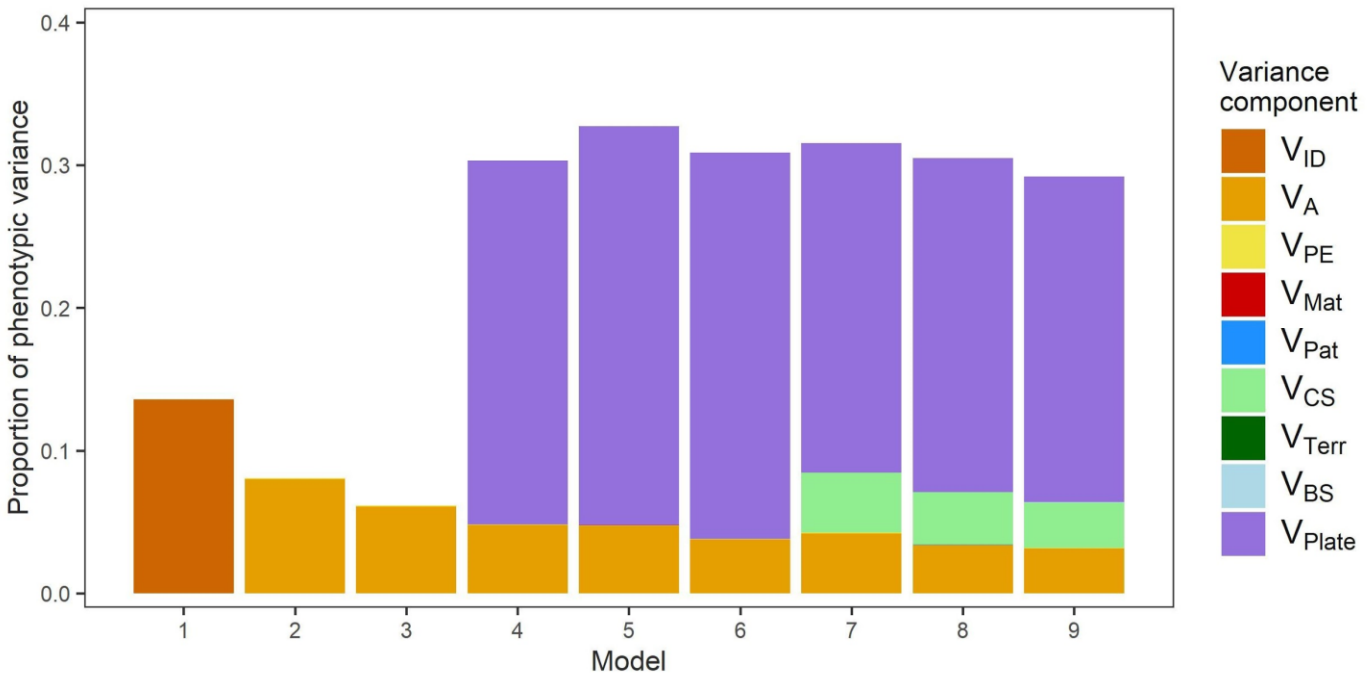
862
863 **Figure 1.** Scatterplots of raw relative telomere length data from the Seychelles warbler showing
864 significant negative within-paternal age at conception effects (A) and positive between maternal
865 age at conception effects (B) on offspring telomere length across all ages (2361 RTL measures 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

Supplemental Information for:

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

Alexandra M. Sparks, Lewis G. Spurgin, Marco van der Velde, Eleanor A. Fairfield, Jan Komdeur, Terry Burke, David S. Richardson, Hannah L. Dugdale

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Parentage Analyses Methods

We used data from the long-term Seychelles warbler database from 1991–2015.

Genotypes

We extracted DNA from red blood cells using either a modified ammonium acetate protocol (Bruford *et al.* 1998; Richardson *et al.* 2001) or, for birds caught from 2013 onwards, a Qiagen DNeasy blood and tissue kit (Qiagen, Crawley, UK). We genotyped 1,853 individuals for up to 30 microsatellite loci and three sexing markers (Table S1), and we characterized these microsatellites using Cervus 3.0.7 (Kalinowski *et al.* 2007). Prior to 2014, samples were molecularly sexed using P2P8 (Griffiths *et al.* 1998) on an agar gel, but from 2014 onwards sexing markers were amplified with the microsatellite markers in multiplexes. The microsatellite and sexing markers were run in 4 multiplexes, with each PCR containing: 5µl Qiagen multiplex-mix (Qiagen Inc., Valencia, USA), 2 µl ddH_2O, 1 µl primer-mix (marker-specific concentrations are provided in Table S1) and 2 µl DNA from ammonium acetate extractions. For DNA extracted using the Qiagen DNeasy kits, the PCR conditions differed slightly, such that either 0.25 µl (multiplexes A1 & B1) or 0.5 µl (multiplexes A2 & B2) primer-mix was used, with 2.75 µl or 2.5 µl ddH_2O, respectively. The PCR-program was as follows: 15 min at 95°C, then 35 cycles for 30s at 94°C, 90s at 55°C (multiplexes A1 & B1) or 56°C (multiplexes A2 & B2), and 60s at 72°C, followed by 30 min at 60°C. PCRs were either run on an ABI 377 or 3730 DNA Analyser. We ran 48 samples on both the ABI 377 and 3730 (Applied Biosystems, California, USA) to ensure consistency of scoring, which resulted in the pooling of alleles 278 and 279, and alleles 283 and 284 at Ase48 and alleles 130 and 131 at Ase9 as these could not be distinguished reliably on the ABI377. We scored the alleles using a ROX500 size-marker and either Genotyper 2.5 or Genemapper 3.7 (Applied Biosystems).

In addition to the 1,853 genotyped birds, 45 candidate parents were not genotyped, as we had no DNA sample for these (N = 43) or the DNA sample failed to amplify (N = 2); however, their phenotypic data were used in the parentage assignments. Including the 45 ungenotyped individuals, the mean proportion of loci typed was 0.971. By re-PCRing DNA from 104 individuals, we calculated the mean allelic drop-out rate as 0.018 (95% CI: 0.012–0.023) and the mean stochastic genotyping error rate as 0.003 (95% CI: 0.001–0.004), using Pedant 1.0 (Johnson & Haydon 2007). Of the total of 1,898 birds, 1,841 were molecularly sexed, and the remaining 57 were sexed behaviourally as we had no remaining DNA sample (N = 47) or the sexing marker failed to amplify (N = 10).

We tested for deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium using GenePop 4.2 (Rousset 2008) with 1,000 dememorizations, 100 batches and 1,000 iterations per batch. Seychelles warblers have a mean life expectancy of 5.5 yr (Komdeur 1991); therefore, we ran the analyses every six years (2015, 2009, 2003, 1997, 1991) using randomly selected genotypes from 26 candidate parents. Due to over-lapping generations, we first removed pairs of candidate parents that we identified as most likely to be first-order relatives, using Related 1.0 (Pew *et al.* 2015), to reduce deviations from the presence of relatives. No genotype was included more than once. Five loci deviated from HWE (1991: Ase16, p = 0.033, SE = 0.003; 1997: Ase22, p = 0.0496, SE = 0.001 and Pdoµ6, p = 0.019, SE = 0.002; 2003: Ase27, p = 0.024, SE = 0.002 and Ase9, p = 0.039, SE = 0.002; 2009: Ase27, p = 0.034, SE = 0.002); however, no loci consistently deviated in more than two years. We controlled for false discovery rate (Benjamini & Hochberg 1995) in the linkage disequilibrium tests, due to the large number of pairwise loci comparisons (N = 435, $\langle$ = 0.05, adjusted p-value = 0.0500–0.0001). Four pairs of loci were in linkage disequilibrium in certain years:

Ase25 & Ase19 (1991: $p < 0.00001$, SE < 0.000001), Ase13 & Ase48 (1997: $p = 0.00005$, SE = 0.00005), Ase13 & Ase56 (1991: < 0.00001, SE < 0.000001), Ase38 & Ase55-cest (2003: $p <$ 0.00001, SE < 0.000001), but no loci were in linkage disequilibrium in more than one year. Using a BLAST search against the zebra finch taeGut3.2.4 in ENSEMBL 83 (Flicek *et al.* 2014), no pairs were assigned to the same chromosome. We therefore included all 30 microsatellites in our analyses.

Offspring birth period and natal territory

We identified the field period (season) of birth of 1,809 offspring from a combination of processing them in the nest ($N = 397$), their eye colour at first capture ($N = 1,399$; eye colour transitions with age (Komdeur 1991): grey eyes < 5 months old, light brown = 5–12 months old, red brown = min 1 year old), or for birds that were independent but no eye colour was recorded at first capture they were assumed to be one year old ($N = 13$).

Blood samples were available from 1993 onwards. Prior to 1993, birds were rung on Cousin in every year from 1980–1991 (there was no fieldwork in 1992). Based on eye colour at first capture in 1993, the oldest birds with red brown would be assumed to be one-year old; therefore, we tested the parentage of offspring estimated to be born from 1992 onwards.

Offspring were then assigned to the field period (i.e. season when researchers were in the field, during which territory membership and social status were assessed, usually in the south-east [Jan–Feb] or north-west [Jun–Oct] monsoon) in which either their date of birth fell or the field period in which the number of days from their date of birth to the middle of the field period was smallest. If the offspring had a social status (i.e. was repeatedly observed in a territory and their status was determined based on their behaviour) in this field period, they were assigned to the territory in which they had a status ($N = 892$), otherwise the territories in which they had a status in the closest field period was used ($N = 917$).

Candidate parent selection

Individuals were defined as offspring in the field period they were identified to have been born in and were excluded as candidate parents for this period. Individuals were then defined as candidate parents in each field period after their natal field period, up to one year after they were last observed. One year was chosen because the annual resighting probability of adult Seychelles warblers on Cousin Island (1986–2004) was high (ca. 0.92 ± 0.02 for birds ≤ 2 years and 0.98 ± 0.01 for older birds (Brouwer *et al.* 2010)). At the end of each field period a social status list is created, in which each bird is listed in the territory that it belongs to, along with its dominant or subordinate status. Candidate parents were defined as dominant pair-bonded or subordinate individuals (Richardson *et al.* 2005).

Statistical analyses

Parentage assignments were run using the Bayesian R-package *MasterBayes* 2.52 (Hadfield *et al.* 2006) in R 3.2.2 (R Core Team 2017). If the territory to which an individual belonged in any field period was unclear (e.g. floaters), the individual was assigned to every territory in which it was observed in that field period. The 'lag' and 'lag_relational' functions were then applied in *MasterBayes* to allow for individuals with multiple locations within a given field period. Locus

specific genotyping error rates were specified (Table S1), along with the allele frequencies from all genotypes. We imposed no restriction on the maximum number of mismatching alleles tolerated between a parent and offspring. The numbers of unsampled dams and sires were estimated by *MasterBayes* in each analysis. When necessary tuning parameters were specified to ensure that the Metropolis-Hasting acceptance rates ranged between 0.2 and 0.5 (Annavi *et al.* 2014). Similarly, the number of iterations was increased to 130,000, discarding the first 30,000 and retaining every 100, to ensure that autocorrelations between successive parameter estimates were < 0.1. Both parents were sampled simultaneously in all analyses. We ran the parentage analyses in three steps, assigning parentage over all years in each analysis.

Step 1: Parentage analysis with restricted maternity

We first ran a parentage analysis for the 1,809 offspring in which maternity was restricted to candidate mothers that had a social status in the offspring's natal territory in the offspring's natal field period. The social status of females is not a significant predictor of maternity assignment (Hadfield *et al.* 2006), but as nearly all paternities are gained by dominant males (Richardson *et al.* 2001; Hadfield *et al.* 2006), we included social status as a predictor of paternity. The status categories were: dominant, subordinate, unknown, and potentially dead (individuals included for one year after their last sighting). During each field period, all territory boundaries were mapped and the centre point of each territory was calculated using ArcGIS 9.3. These centre points were then used in the parentage analyses to restrict candidate mothers to females residing in an offspring's natal territory, and to weight the probability of paternity by the Euclidian distance in meters between the offspring's and candidate father's territory, as the probability of paternity declines with distance (Hadfield *et al.* 2006). We also included age and age-squared of both the candidate mothers and fathers as parentage predictors, as Seychelles warblers undergo senescence (Hammers *et al.* 2012; 2015).

The annotated R code for the first step in the parentage analysis is provided at the end of this supplementary parentage materials.

Step 2: Parentage analysis with unrestricted maternity

In the second step, we re-ran the parentage analyses for all offspring that were not assigned a mother with ≥ 0.80 probability in the first step (N = 606). For these offspring, we removed the restriction that candidate mothers must reside in the offspring's natal group in the field period that they were born in. This was to account for individuals that were not caught in their natal period and therefore their first record may be in a non-natal territory. Parentage was run in three analyses where:

2a) The probability of maternity and paternity were weighted by the distance of the candidate mother or father, respectively, to the offspring's natal territory. Although maternity was not restricted to the natal group, distance between candidate mothers and offspring was modeled, as natal dispersal distance is generally over a short distance (females = median of 4 territories; males = 2); therefore, offspring that had dispersed are likely to be in the vicinity of their natal group (Eikenaar *et al.* 2008).

2b) Only the probability of paternity was weighted by distance.

2c) No weighting on distance.

In total, we assigned mothers to 317 offspring with ≥ 0.80 probability in analyses 2a, 2b, or 2c. No offspring were assigned a different mother with ≥ 0.80 probability in analyses 2a, 2b, or 2c.

Step3: Parentage analysis with informed maternity restriction

In the final parentage analysis, we re-ran the analyses from step1, but the mother's territory, of the 317 offspring assigned a mother in step 2 with ≥ 0.80 probability, was used as their natal territory. Thus, we re-ran the parentage analysis as if the offspring had originally been assigned to that territory, which has ramifications for the assignment weightings for paternity.

Pedigree statistics were generated using Pedantics 1.5 (Morrissey & Wilson 2010) and Pedigree Viewer 6.5b (Kinghorn 1994). When the most-likely father had a social status in more than one territory, the offspring was assigned as a within-group offspring if the father had a status in the offspring's natal territory; otherwise, it was scored as an extra-group offspring.

Parentage Analyses Results

We assigned mothers to 1,487 offspring and fathers to 1,554 offspring, with ≥ 0.80 probability; 255 offspring had no parents assigned. Overall these assignments had high confidence with 93% assigned with ≥ 0.95 probability (1,448 fathers and 1,379 mothers). The intensity of fieldwork increased dramatically from 1997 and 65% of the offspring with an unassigned parent were born before 1997 (Fig. S1). Only 4% of the assigned mothers or fathers had any mismatching alleles with their offspring, or 11% of the trios, with a maximum of three mismatching alleles occurring (Table S2). The pedigree contains 10 generations (mean depth = 3.4 [95% CI = 3.3–3.6]; Table S3 and Fig. S2).

The posterior mode of the number of unsampled fathers per field period on Cousin was 6.8 (95% CrI: 5.1–9.0) and of unsampled mothers within an offspring's natal territory was 0.07 (95% CrI: 0.06–0.09).

Age had a negative quadratic effect on both the probability of paternity and maternity assignment (Fig. S3), consistent with the occurrence of senescence in this species. The probability of paternity decreased with the distance between a candidate father and an offspring (Fig. S3). Using parentage assignments with at least 0.80 probability, the most-likely fathers of 44% of the offspring were extra-group males.

Dominant males had a higher probability of gaining paternity than males of subordinate, unknown or potentially dead status (Fig. S3). Only 49 subordinate males were assigned paternity: 22 were within-group and 27 were extra-group fathers. However, 12/27 extra-group subordinate fathers became a dominant male in the following season; thus, they are likely to have been transitioning to dominant status when they gained paternity. Twelve of the remaining 15 subordinate extra-group fathers were assigned paternity with ≥ 0.95 probability. Four of the offspring fathered by these subordinate extra-group males did not have a social status in their natal field period as they were first caught as independents; three had their natal group assigned in step 2 of the parentage assignments. Excluding these four, there were therefore only eight extra-group subordinate fathers assigned with high confidence (0.05% of assigned fathers). Similarly, for the within-group subordinate fathers, five were assigned with <0.95 probability, six became dominant males in the next season and nine were

assigned to offspring that were first caught after independence, leaving only two within-group subordinate fathers assigned with high confidence (0.01% of assigned fathers).

Dominant females accounted for 85% of the maternity assignments (1,263/1,487 offspring), with the remaining offspring assigned to females of subordinate (11%; N=164), unknown (2%; N=30) and potentially dead (2%; N=30) status.

Parentage R Code

```
library(MasterBayes)
# Read the genotype and phenotype files:
WarbP<-read.table("WarbP_20150829_ForR.txt",header=T,na.strings="NA")
WarbG<-read.table("WarbG_20150902.txt",header=T,na.strings="NA")
# Restrict candidate mothers to those with a social status in an offspring's natal territory in the field period it was born in:
res1<-expression(varPed(x="Terr", gender="Female", relational="OFFSPRING", restrict="==",lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Exclude offspring as parents:
res2<-expression(varPed(x="offspring", gender=NULL, relational=FALSE, restrict=0, lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Model the probability of paternity as a function of the Euclidian distance between a candidate father and offspring:
var1 <- expression(varPed(x = c("Lat", "Longitude"), gender="Male", relational="OFFSPRING", lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Model the probability of paternity of a function of male social status:
var2 <- expression(varPed(x = "UniqueSub_Dom", gender="Male", lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Model the probability of paternity of a function of male age:
var4 <- expression(varPed(x="Age_Int", gender="Male", lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Model the probability of maternity of a function of female age:
var5 <- expression(varPed(x="Age_Int", gender="Female", lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Model the probability of paternity of a quadratic function of male age:
var6 <- expression(varPed(x="Age_Int2", gender="Female", lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Model the probability of maternity of a quadratic function of female age:
var7 <- expression(varPed(x="Age_Int2", gender="Male", lag=c(-0.006,0.006), lag_relational=c(-0.006,0.006)))
# Create the PdataPed object, which contains the phenotype data:
PdP_var124567 <-PdataPed(formula=list(res1, res2, var1, var2, var4, var5, var6, var7), data=WarbP, timevar=WarbP$UniqueTimeVar, USsire=TRUE, USdam=TRUE)
```

```
# Create the GdataPed object, which contains the genotype data:
GdP<-GdataPed(G=WarbG, perlocus=TRUE)
# Read in the locus specific error rates:
error <- read.table("error.txt",header=T)
# Define the starting parameterisations:
sP<-startPed(estG=FALSE, A=extractA(WarbG), E1=error$e1, E2=error$e2, estUSsire=TRUE,
estUSdam=TRUE)
# Define the scaling constants for the tuning parameters, so that the Metropolis-Hastings acceptance
rates range between 0.2 and 0.5:
tP <-tunePed(beta=c(0.15,0.15,0.15,0.15,0.15,0.15,0.15,0.15), USdam = 0.2, USsire = 0.2)
# Run the first step of the parentage analysis:
model4jn_var124567<-MCMCped(PdP=PdP_var124567, GdP=GdP, sP=sP, tP=tP,
write_postP="JOINT", jointP=TRUE, verbose=TRUE, nitt = 130000, thin=100, burnin=30000)
#  Ensure that successive samples from the posterior distribution have low autocorrelation (<0.1):
autocorr(model4jn_var124567$USdam)
autocorr(model4jn_var124567$USsire)
autocorr(model4jn_var124567$beta)
autocorrP(model4jn_var124567$P)
```

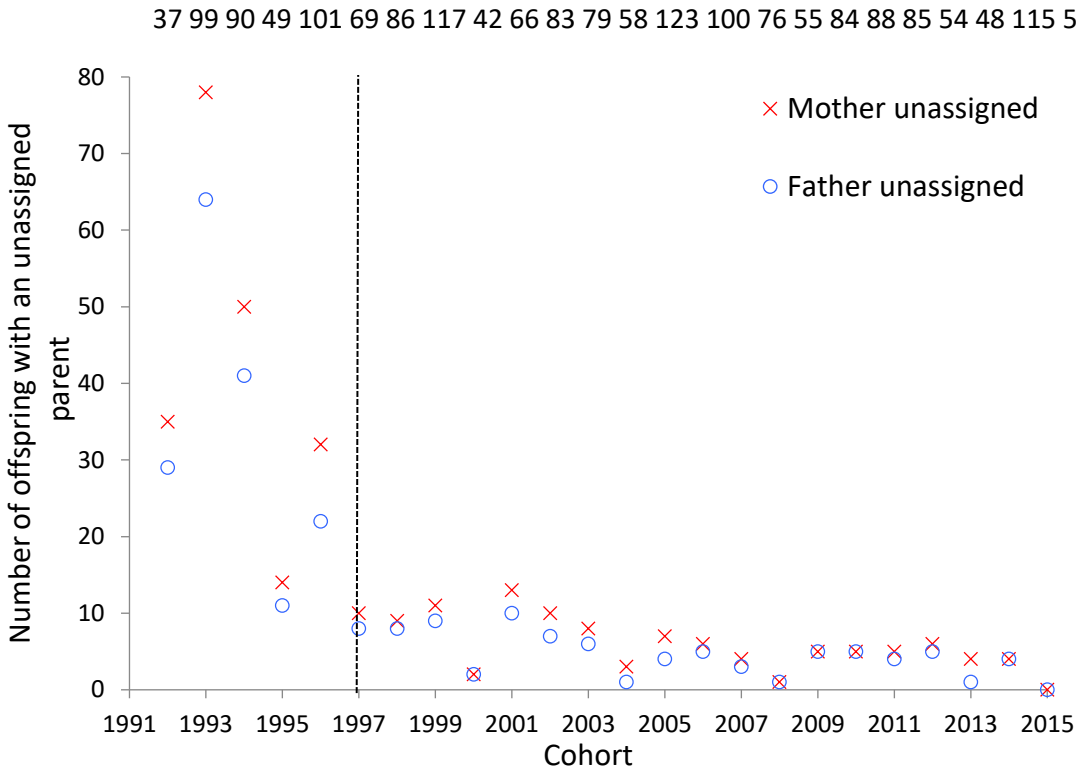
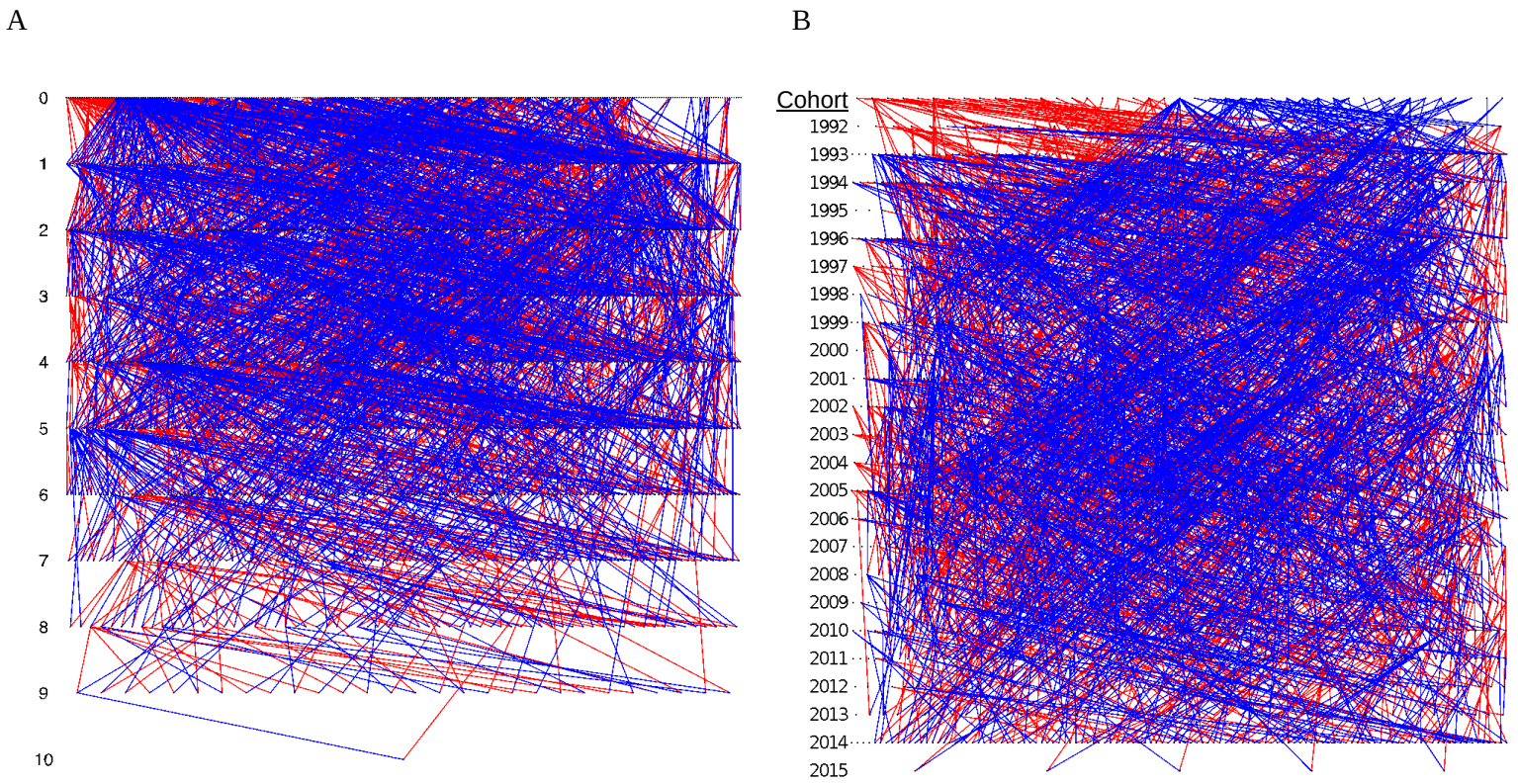


Figure S1 The number of offspring with an unassigned mother or father according to their year of birth (cohort). The dashed line indicates that intensive fieldwork commenced in 1997. Numbers at the top of the graph represent the number of offspring that parentage was tested of in each cohort.

1145



1146 **Figure S2** The Cousin island Seychelles warbler pedigree (1992–Feb 2015) plotted: (A) per generation and (B) per cohort (the top cohort
1147 represents individuals born before 1992 for whom parentage was not tested). Red lines link mothers and offspring, and blue lines (which
1148 overlay the red lines) link fathers and offspring. Dots represent individuals.

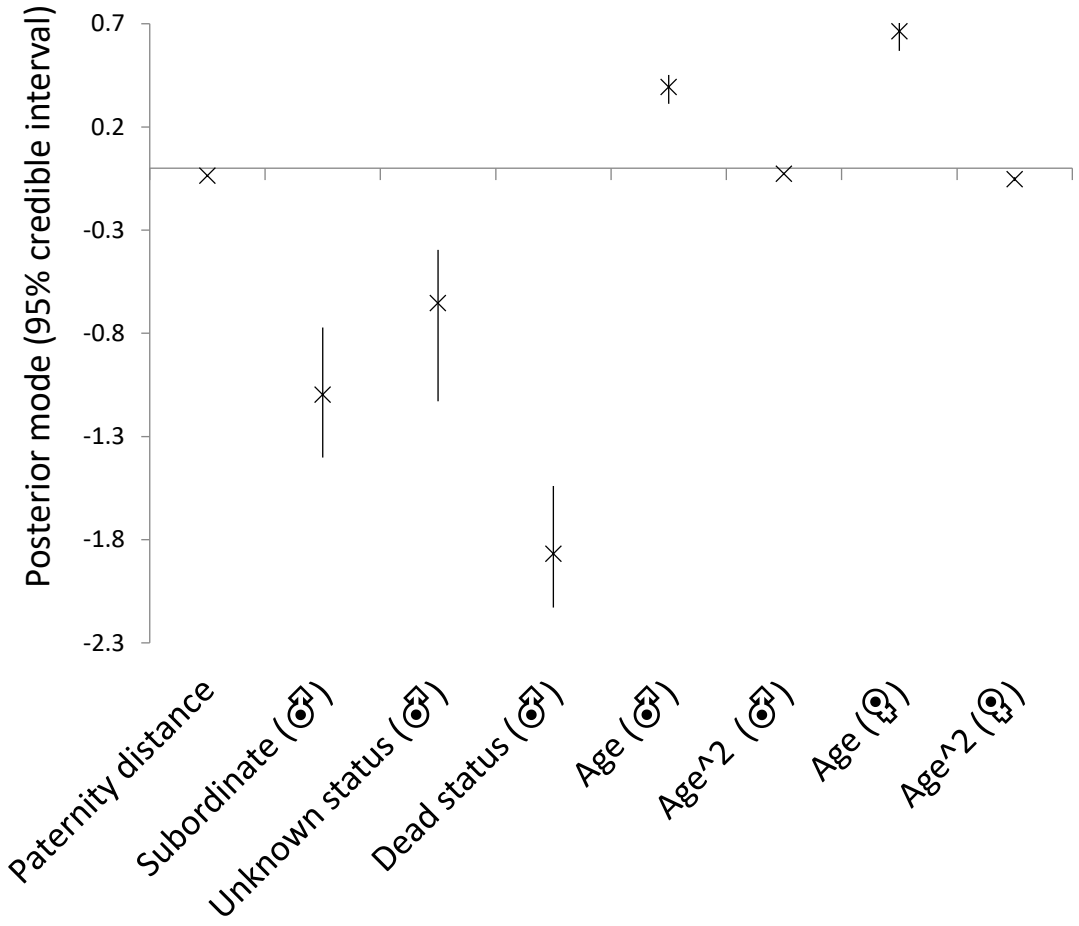


Figure S3 Posterior density estimates, and their 95% credible intervals, of the parameters from the parentage assignment model (step 3). The social statuses of subordinate, unknown and potentially dead are plotted relative to dominant.

1153 **Table S1** Characterization of 30 microsatellite and three sexing markers in the Cousin Island population of Seychelles warblers

Locus	GenBank Accession Number	Forward sequence (5'-3')	Reverse sequence (5'-3')	Primer conc. (μM)†	Label	Multiplex	*k*	*N*	Min allele size (bp)	Max allele size (bp)	H_o	H_e	*PIC*	*HW*	F_{null}	*e1*	*e2*	Reference
Ase3	AJ287386	ACAGGTATGGCGCTCAAGTC	CTGAATCTTACACAGGAGACCGT	0.04	HEX	B2	3	1840	94	100	0.448	0.455	0.374	NS	0.0082	0.02822	0	(Richardson *et al.* 2000)
Ase4	AJ287387	TCTCCATCATCACCACAAAGC	TTCCCATTGCCCTAGTTATTCCA	0.1	FAM	B1	2	1851	106	108	0.461	0.45	0.348	NS	-0.0124	0.00859	0.00709	(Richardson *et al.* 2000)
Ase6	AJ287389	TAAAAGCCAGCAGTGGAGCC	CGAGCTTGCAGGGTTTCCT	0.1	FAM	B1	4	1850	117	129	0.704	0.7	0.643	NS	-0.0032	0.00730	0.00640	(Richardson *et al.* 2000)
Ase7	AJ287390	AATCAACTTCAAATGCTCACAG	ACTACATGACTCCAGGCTCAG	0.025	FAM	A2	2	1837	118	122	0.493	0.471	0.36	NS	-0.0224	0.02281	0	(Richardson *et al.* 2000)
Ase9	AJ287392	GACTGAAGTCCTTTCTGGCTTC	CACCAGGAATACAAGTCCATTG	0.1	NED	A1	3	1852	130	137	0.447	0.46	0.403	NS	0.0143	0	0	(Richardson *et al.* 2000)
Ase10	AJ287393	CATTGGGGTACTATGGAAAGACC	TCCTGAGTGGAAGGAACATAGG	0.1	FAM	A1	3	1852	122	143	0.444	0.434	0.38	NS	-0.0117	0.02252	0	(Richardson *et al.* 2000)
Ase11	AJ287393	TCCCCAAATCTCTCAATTCC	AGTTCTAAGCCTGCCTGTGC	0.03	NED	B2	3	1840	115	127	0.5	0.511	0.413	NS	0.0116	0.02125	0	(Richardson *et al.* 2000)
Ase13	AJ287396	TGTGCTCCTCTGCTTTCC	CAGATGGCCAGTGTTAGTCC	0.1	HEX	B1	3	1852	140	152	0.505	0.505	0.435	NS	-0.0011	0.01107	0.00681	(Richardson *et al.* 2000)
Ase16	AJ276374	TCAGTTCCTGAGTAAATGTCTC	TGAATTACCCCTAAATACCTG	0.3	HEX	B2	8	1834	151	184	0.722	0.74	0.702	NS	0.0128	0	0.01699	(Richardson *et al.* 2000)

Ase18	AJ276375	ATCCAGTC TTCGCAAA AGCC	TGCCCCAG AGGGAAG AAG	0.1	HEX	B1	4	1851	184	196	0.5	0.499	0.402	NS	-0.0021	0.01133	0.00670	(Richardson *et al.* 2000)
Ase19	AJ276376	TAGGGTCC CAGGGAGG AAG	TCTGCCCA TTAGGGAA AAGTC	0.025	FAM	A2	2	1841	171	177	0.496	0.493	0.372	NS	-0.0027	0.01999	0	(Richardson *et al.* 2000)
Ase22	AJ276379	TGAACCAT TGTCACCA ACAC	GCTTTAGT TCAGATGC CCAG	0.04	NED	A2	2	1840	179	183	0.391	0.39	0.314	NS	-0.0016	0.02560	0	(Richardson *et al.* 2000)
Ase25	AJ276382	GATGGCTA TATGCTTCA AATGC	TTGAAAGC CTTAAAGT GGGA	0.1	FAM	B1	8	1849	173	217	0.703	0.714	0.668	NS	0.0086	0.01790	0	(Richardson *et al.* 2000)
Ase27	AJ276384	TTAACATT GCATGCTC CTGC	AGTCAAGG TACAGGCT AGATAGCC	0.1	NED	A1	8	1849	184	230	0.643	0.645	0.575	NS	0.0009	0	0.01163	(Richardson *et al.* 2000)
Ase35	AJ276637	GTCCTTGGT CCTTAGCA TCTGT	GCTCCTGT TGTTCTGG GAATAG	0.05	NED	B1	3	1852	230	234	0.585	0.585	0.508	NS	-0.0019	0.01840	0	(Richardson *et al.* 2000)
Ase37	AJ276639	TAATTCAT GGAGAAGC CCAG	TCAAAACA ACAGTTTT CACAGC	0.4	FAM	A1	3	1851	237	247	0.401	0.413	0.373	NS	0.0192	0.02476	0	(Richardson *et al.* 2000)
Ase38	AJ276640	ATCCGAGA ACCCAATC ACTT	GCAGCATT ACAGTCTC AAAGAAC	0.04	NED	B2	2	1839	224	228	0.452	0.462	0.355	NS	0.0108	0.01512	0	(Richardson *et al.* 2000)
Ase42	AJ276644	CATGGGTA GGTTGGGA TGTC	AGGTGAGG GTATGCAA ACATG	0.05	NED	A1	2	1849	249	253	0.269	0.259	0.226	NS	-0.0191	0.07292	0	(Richardson *et al.* 2000)
Ase48	AJ276777	TTTATTTCC TGGACTGG AACAATC	GAACATTG GGCTACTG GGC	0.2	FAM	A1	3	1848	272	284	0.53	0.537	0.433	NS	0.0062	0.01999	0	(Richardson *et al.* 2000)
Ase53	AJ276782	ATGGAGAA TTCTGGGT GCTG	CCCAATAA TGAGGTAA CACCAA	0.05	FAM	B2	5	1836	266	287	0.525	0.546	0.474	NS	0.0204	0.01116	0	(Richardson *et al.* 2000)

Ase55-cest	-	AGCTGGATTGGCATCGTG	TCATTACAGCAATTACCATTGAGC	0.05	HEX	A2	2	1837	278	280	0.402	0.426	0.335	NS	0.0292	0.04712	0	(Dawson *et al.* unpublished data)
Ase56	AJ276785	TTCACTGAGAAGTGAGAATGTG	GTCCTTGATTGATTACAGGCT	0.2	FAM	B1	3	1849	299	305	0.399	0.413	0.364	NS	0.0168	0.01903	0	(Richardson *et al.* 2000)
Ase58	AJ276787	ATTCCAGGGATTGGGCAG	CTCAAAGCGAAATTGAGCAGT	0.2	HEX	A1	7	1847	283	323	0.721	0.729	0.691	NS	0.0043	0.00621	0.00645	(Richardson *et al.* 2000)
Ase61	AJ276790	AGGATTTTTAATGGGATATACACATCTG	AGCCACATTTTAGCCCACAG	0.1	HEX	A2	8	1831	355	390	0.538	0.545	0.456	NS	0.007	0.01734	0.00371	(Richardson *et al.* 2000)
Ase64	AJ276793	CCACCTTTCATACTGGGGAG	TTCAGCCAGTCAGTGTAGCC	0.05	HEX	A2	3	1832	405	413	0.621	0.625	0.546	NS	0.0024	0.00821	0.00698	(Richardson *et al.* 2000)
Calex-08-gga	AM072456	TTAMAGAATTCTTTCACATGGTCTCT	GTTTCTTCGGAATATTAAGTAGAGGCTTCCAT	0.1	HEX	B2	2	1834	344	348	0.16	0.155	0.143	NS	-0.0145	0.02167	0	(Kupper 2008)
Cuu4-gga5	AF122891	CRKGCAAGMACAAAGCAAAATCC	TTCCTAAAYCTCARRTKGACTCAAG	0.05	HEX	A2	2	1836	234	238	0.449	0.452	0.35	NS	0.0037	0.01481	0	(Martín-Gálvez *et al.* 2009)
Pdoμ6	Y15125	CTGATCATGTGTAGATGTAAGACTGC	CAGATCCTTAAGCAGGAAGTTAGG	0.05	FAM	B2	3	1836	205	212	0.556	0.539	0.463	NS	-0.0169	0.00767	0.00787	(Griffith *et al.* 1999)
PmaTGA42	AY260540	ACTTCCACATGCCAGTTTCC	TGTTAAGGCAGAGAGGTGGG	0.05	FAM	A2	3	1835	256	264	0.495	0.492	0.371	NS	-0.0033	0.00891	0	(Saladin *et al.* 2003)
Pte24-cest	-	AACAAAGGACGCCGAGTAG	TCATTTAATGGCTYTA	0.05	HEX	B2	2	1838	239	241	0.029	0.029	0.029	ND	0.011	0.01589	0	(Dawson *et al.*

			CTTCATACAT														unpublished data)
P2P8	-	TCTGCATCGCTAAATCCTTT	CTCCCAAGGATGAGRAAYTG	0.1	FAM	A1	2	1841	356	387	n/a	n/a	n/a	n/a	n/a	n/a	n/a	(Griffiths *et al.* 1998)
zoo2A	-	-	-	0.03	FAM	A2	2	490	213	222	n/a	n/a	n/a	n/a	n/a	n/a	n/a	(Dawson 2007)
zoo2B	-	-	-	0.15	FAM	B1	2	494	240	249	n/a	n/a	n/a	n/a	n/a	n/a	n/a	(Dawson 2007)

†Primer concentrations are based on using the ammonium acetate DNA extractions where were 1µl primer-mix was used. For Qiagen DNA extractions, primer concentrations were 2–4 times lower; we added 0.25 µl (multiplexes A1 & B1) or 0.5 µl (multiplexes A2 & B2) primer-mix in the PCR reaction.

k = number of alleles

N = number of individuals genotyped

H_o = observed heterozygosity

H_e = expected heterozygosity

PIC = polymorphic information content

e1 = allelic drop-out rate

e2 = stochastic genotyping error rate

HW = significance of deviation from Hardy-Weinberg equilibrium (NS = not significant, ND = not done)

F_{null} = estimate null allele frequency

n/a = not applicable

- = not available

Table S2 Allelic mismatch summary between mother–offspring, father–offspring and mother–father–offspring assigned with ≥ 0.80 probability

Number of mismatches	Mother–offspring	Father–offspring	Trio
0	1,428	1,489	1,329
1	51	57	125
2	4	8	26
3	4	0	7
Total	1,487	1,554	1,487
Percentage mismatching	4%	4%	11%

Table S3 Pedigree statistics using parents assigned with ≥ 0.8 probability for the full pedigree and the pruned pedigree used in this study

Parameter	Full pedigree (N)	Pruned pedigree (N)
records	1853	1482
offspring	1809	1268
maternities	1487	1217
paternities	1554	1268
mothers	420	391
fathers	400	367
full sibs	946	696
maternal sibs	3347	2365
maternal half sibs	2401	1669
paternal sibs	4450	3150
paternal half sibs	3504	2454
maternal grandmothers	1011	860
maternal grandfathers	1082	913
paternal grandmothers	1047	891
paternal grandfathers	1151	975
maximum pedigree depth†	10	10
founders	299	214
mean maternal sibsip size	3.540	3.113
mean paternal sibsip size	3.885	3.455
non-zero F	265	210
F > 0.125	56	41
mean pairwise relatedness	0.009	0.011
pairwise relatedness>=0.125	0.026	0.030
pairwise relatedness>=0.25	0.010	0.012
pairwise relatedness>=0.5	0.002	0.003

†Where the baseline generation is generation 0

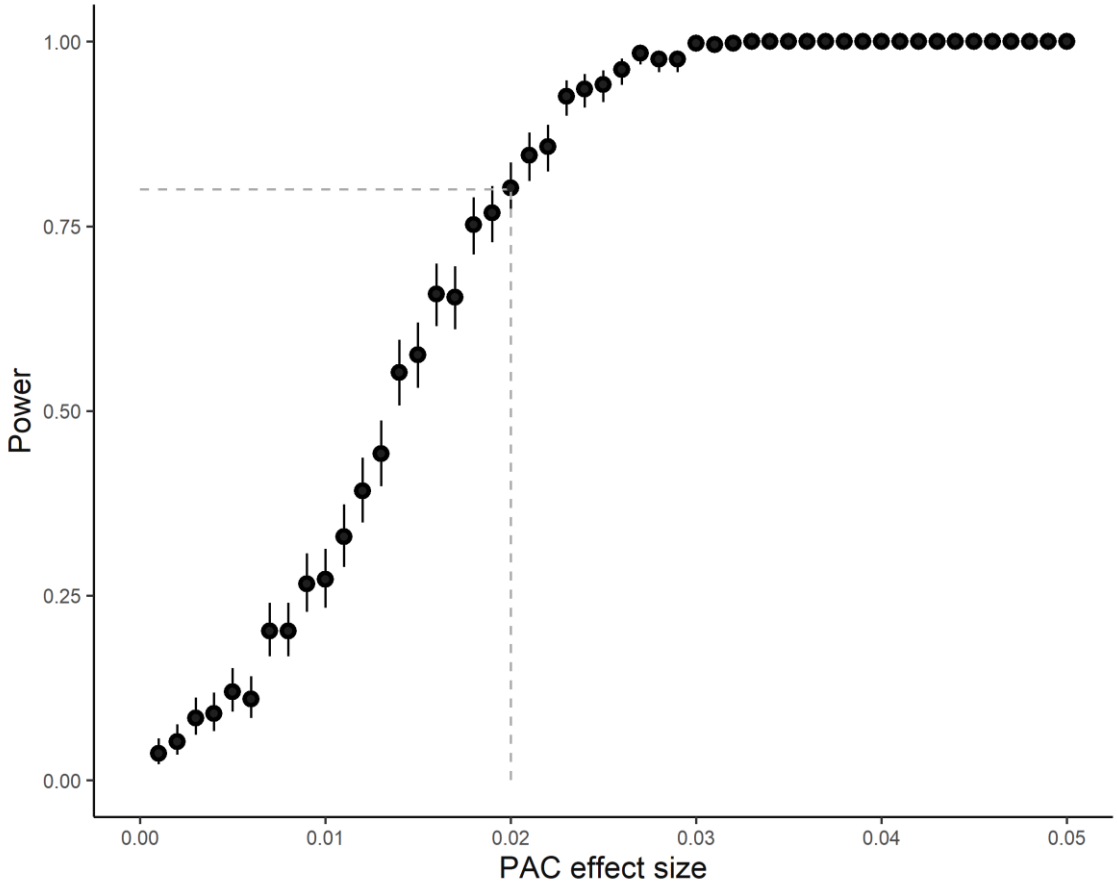


Figure S4. Power for detecting paternal age at conception (PAC) effect sizes in the Seychelles warbler, using measures from the dataset with all ages and the given model structure (see Methods), run in *simr* 1.0.5 (Green and MacLeod 2016). Points and bars show the mean power and 95% confidence intervals, respectively, based on 500 simulations. Grey dashed line indicates effect sizes that can be determined with a power of ≥80%. We have power to detect PAC effect sizes of ≥0.02, equivalent to a correlation coefficient of 0.059 (see Methods).

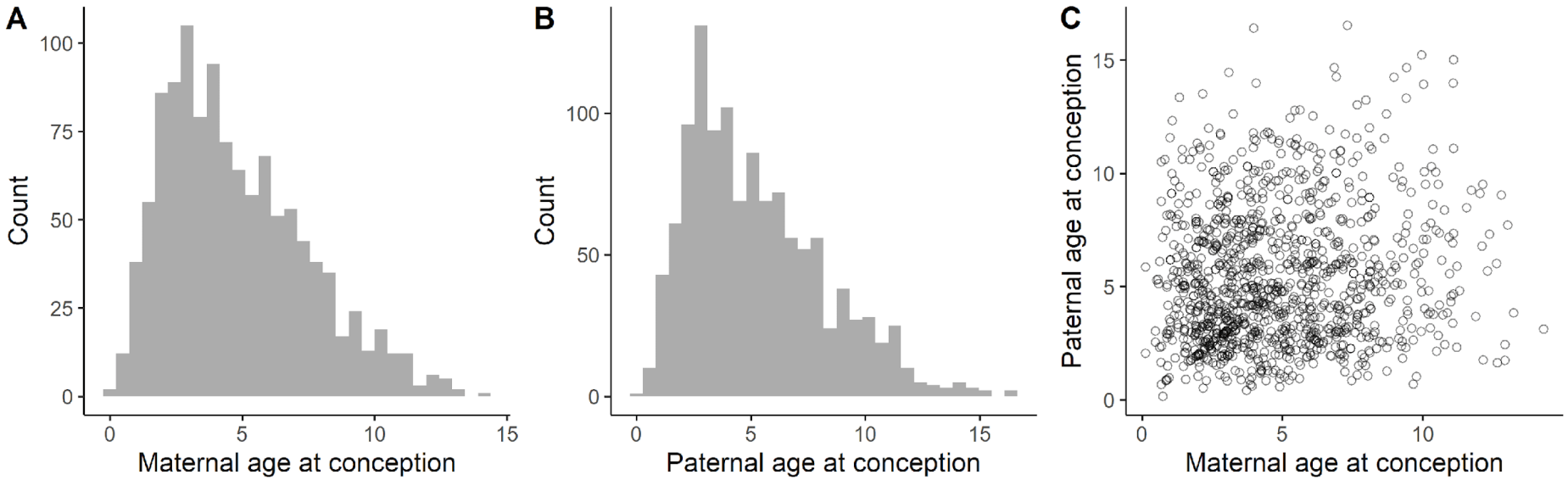


Figure S5. Histograms of the frequency of maternal (A) and paternal (B) ages at conception in the Seychelles warbler, and (C) a scatterplot showing the correlation between maternal and paternal ages at conception for offspring in the dataset (n=1318 offspring, 380 mums and 354 dads).

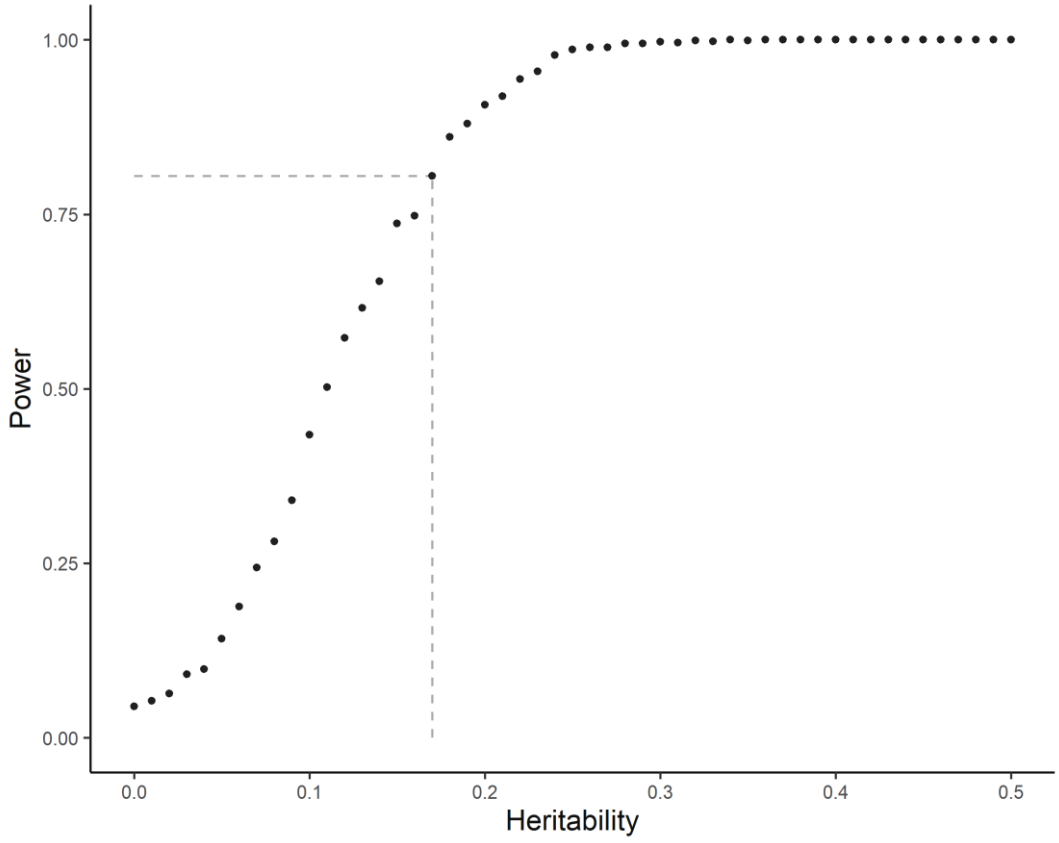


Figure S6. Power analysis of our ability to detect a range of heritability estimates of telomere length using the Seychelles warbler pruned pedigree and dataset. Power was calculated using *Pedantics* 1.7 (Morrissey & Wilson, 2010) based on 1000 simulations. Points show the mean power and the dotted line indicates 80% power. The pruned pedigree had ≥80% power to detect telomere length heritabilities of ≥0.17 in this dataset.

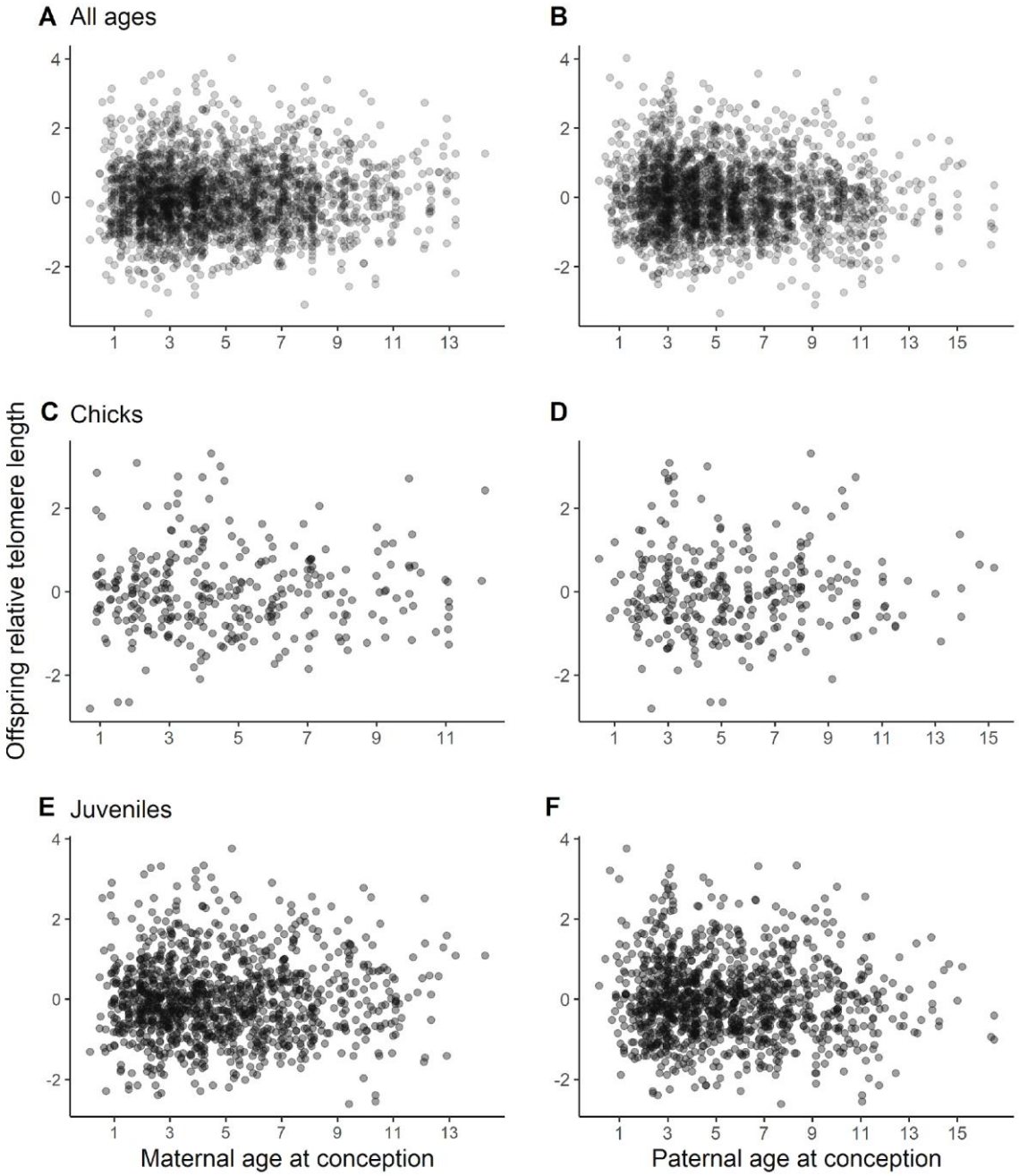


Figure S7. Scatterplots of raw relative telomere length data from the Seychelles warbler showing associations between maternal (A, C, E), and paternal (B, D, F) age at conception on offspring relative telomere length when telomeres were measured in offspring in all ages (A, B; n=2361 measures of 1156 offspring), or taking just the first chick measure (C, D; n=304 offspring) or all juvenile (<1 year old) measures (E, F; n=1137 measures of 958 offspring). Full model results are provided in Table S5.

Table S4. Heritability estimates of relative telomere length in the Seychelles warbler estimated using parent–offspring regressions. Heritabilities were calculated from maternal–offspring, paternal–offspring and midparent–offspring regressions, using mean RTL of the offspring and parent, as well as models where the RTL measure was taken as juveniles (<1 year) (see offspring measure–parent measure). Where parents had multiple offspring, a mean of the mean RTL from their offspring was taken. Included are the estimated effects (estimate), standard error (SE), sample size (n), significance based on the t-value (P) and heritability estimates. Plots of raw data are in Figure 2.

Offspring measure–parent measure	Maternal–offspring					Paternal–offspring					Midparent–offspring				
	estimate	SE	n	P	h^2	estimate	SE	n	P	h^2	estimate	SE	n	P	h^2
Mean – Mean	0.050	0.043	303	0.246	0.101	0.018	0.049	284	0.715	0.035	0.096	0.060	585	0.112	0.096
Juvenile – Juvenile	0.064	0.054	172	0.241	0.128	0.007	0.061	165	0.911	0.014	0.094	0.088	210	0.285	0.094

Table S5. Linear mixed model results of the association of maternal and paternal age at conception (MAC and PAC, respectively) on offspring telomere length in the Seychelles warbler. Associations were investigated in offspring telomere length of all ages, when offspring measurements were taken as chicks (first measure) or as juveniles (<1 year old). Obs = number of observations; IDs = number of individuals. 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 and then z-transformed in all models and age was log-transformed for the models of all ages and juveniles, but not for chicks. For plots of raw data see Figure S7. P<0.05 are shown in bold.

	All ages	Obs=2361/IDs=1156			Chicks	Obs/IDs=304			Juveniles	Obs=1137/IDs=958		
variables	estimate	SE	LRT	P-value	estimate	SE	LRT	P-value	estimate	SE	LRT	P-value
fixed effects												
Intercept	-0.132	0.077			0.371	0.226			-0.429	0.103		
Log Age (years)	**-0.297**	**0.030**	**94.767**	**<0.001**	**-12.103**	**4.958**	**5.812**	**0.016**	**-0.448**	**0.054**	**66.225**	**<0.001**
Sex (male)	0.022	0.039	0.310	0.578	-0.065	0.104	0.400	0.527	0.074	0.053	1.933	0.165
Technician	**0.481**	**0.076**	**38.312**	**<0.001**	0.574	0.333	2.961	0.085	**0.443**	**0.123**	**12.809**	**<0.001**
MAC	0.010	0.008	1.780	0.182	0.000	0.020	<0.001	0.992	0.004	0.010	0.167	0.683
PAC	-0.013	0.007	3.280	0.070	0.009	0.021	0.164	0.685	-0.008	0.009	0.715	0.398
random effects												
ID	0.039								0.030			
Mother identity	0.022				0.107				0.023			
Father identity	0.004				0.173							
Plate ID	0.189				0.389				0.212			
Season ID	0.029				0.027				0.017			
Residual	0.655				0.428				0.660			

Table S6. Animal model variance component estimates and their associated proportions of the total phenotypic variance from a MCMC model of relative telomere length in the Seychelles warbler. Results are from models 1–8 (see Methods, Figure 3); the results from model 9 are provided in Table 2. Variance components reported are the following: individual identity (V_{ID}), additive genetic (V_A), permanent environment (V_{PE}), qPCR plate (V_{Plate}), maternal (V_{Mat}), paternal (V_{Pat}), capture season (V_{CS}), current territory (V_{Terr}), birth season (V_{BS}) and residual variance (V_R). 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% CrIs. †indicates models where the fixed effects are included. The proportion of phenotypic variance explained by each variance component in models 1–9 are plotted in Figure 3.

Model	Variance component	Posterior mode	Lower 95% CrI	Upper 95% CrI	Prop V_P	Lower 95% CrI	Upper 95% CrI
Model 1	V_{ID}	0.020	0.012	0.031	0.136	0.078	0.195
	V_R	0.135	0.124	0.144	0.864	0.805	0.922
Model 2	V_A	0.015	0.006	0.023	0.080	0.041	0.144
	V_{PE}	<0.001	<0.001	0.016	0.001	<0.001	0.101
	V_R	0.131	0.124	0.143	0.873	0.799	0.915
Model 3†	V_A	0.009	0.003	0.016	0.061	0.024	0.113
	V_{PE}	<0.001	<0.001	0.015	0.001	<0.001	0.100
	V_R	0.127	0.118	0.137	0.893	0.831	0.940
Model 4†	V_A	0.006	0.003	0.012	0.048	0.017	0.081
	V_{PE}	<0.001	<0.001	0.009	<0.001	<0.001	0.061
	V_{Plate}	0.040	0.031	0.051	0.255	0.220	0.323
	V_R	0.098	0.091	0.106	0.651	0.601	0.718
Model 5†	V_A	0.006	0.002	0.012	0.047	0.011	0.077
	V_{PE}	<0.001	<0.001	0.009	<0.001	<0.001	0.059
	V_{Plate}	0.043	0.031	0.050	0.280	0.219	0.320
	V_{Mat}	<0.001	<0.001	0.003	<0.001	<0.001	0.023
	V_R	0.098	0.091	0.106	0.657	0.599	0.717

Model 6†	V_A	0.005	0.001	0.011	0.038	0.008	0.075
	V_{PE}	<0.001	<0.001	0.009	<0.001	<0.001	0.059
	V_{Plate}	0.038	0.032	0.052	0.270	0.217	0.320
	V_{Mat}	<0.001	<0.001	0.004	<0.001	<0.001	0.025
	V_{Pat}	<0.001	<0.001	0.002	<0.001	<0.001	0.011
	V_R	0.098	0.091	0.105	0.654	0.603	0.721
Model 7†	V_A	0.006	0.002	0.011	0.042	0.012	0.075
	V_{PE}	<0.001	<0.001	0.009	<0.001	<0.001	0.059
	V_{Plate}	0.037	0.028	0.047	0.231	0.193	0.295
	V_{Mat}	<0.001	<0.001	0.004	<0.001	<0.001	0.024
	V_{Pat}	<0.001	<0.001	0.002	<0.001	<0.001	0.011
	V_{CS}	0.004	0.002	0.012	0.042	0.013	0.073
	V_R	0.097	0.090	0.104	0.634	0.589	0.709
Model 8†	V_A	0.005	0.001	0.010	0.034	0.006	0.069
	V_{PE}	<0.001	<0.001	0.009	<0.001	<0.001	0.057
	V_{Plate}	0.034	0.028	0.046	0.234	0.194	0.296
	V_{Mat}	<0.001	<0.001	0.004	<0.001	<0.001	0.024
	V_{Pat}	<0.001	<0.001	0.002	<0.001	<0.001	0.012
	V_{CS}	0.006	0.002	0.012	0.037	0.012	0.076
	V_{Terr}	<0.001	<0.001	0.003	<0.001	<0.001	0.018
	V_R	0.096	0.090	0.104	0.650	0.584	0.703

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Table S7. Animal model variance component estimates and their associated proportions of the total phenotypic variance from an ASReml model of relative telomere length in the Seychelles warbler. Structure of the model was equivalent to the *MCMCglmm* model 9 (see Methods, Table 2, Figure 3). Variance components reported are the following: additive genetic (V_A), permanent environment (V_{PE}), qPCR plate (V_{Plate}), maternal (V_{Mat}), paternal (V_{Pat}), capture season (V_{CS}), current territory (V_{Terr}), birth season (V_{BS}), natal territory (V_{NTerr}) and residual variance (V_R). Included are the variance component estimates (Est) and their standard errors in brackets as well as the proportion of the total phenotypic variance explained by the term (Prop) with their associated standard errors in brackets. Significance of random effects was determined by dropping each random effect from a model containing all random effects and performing a likelihood ratio test (LRT) using twice the absolute difference in log-likelihoods between the two models where df=1. [B] indicates where variance components have gone to boundary. P<0.05 are shown in bold.

	Est	Prop	LRT	P
V_A	**0.006**	**0.041**		
	(0.003)	**(0.018)**	**7.914**	**0.005**
V_{PE}	0.002	0.017		
	(0.003)	(0.023)	0.520	0.471
V_{Plate}	**0.032**	**0.222**		
	(0.004)	**(0.025)**	**223.892**	**<0.001**
V_{Mat}	0.001	0.005		
	(0.002)	(0.011)	-52.717	1.000
V_{Pat}	<0.001	<0.001		
	(<0.001)[B]	(<0.001)[B]	N/A	N/A
V_{CS}	**0.005**	**0.034**		
	(0.002)	**(0.014)**	**17.536**	**<0.001**
V_{Terr}	0.001	0.005		
	(0.001)	(0.007)	0.516	0.472
V_{BS}	0.001	0.006		
	(0.001)	(0.006)	0.940	0.332
V_R	0.097	0.671		
	(0.004)	(0.031)	-	-

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