Heritability and age-dependent changes in genetic variation of telomere length in a wild house sparrow population

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Abstract

Telomeres are a popular biomarker of senescence, as telomere dynamics are linked with survival and lifespan. However, the evolutionary potential of telomere dynamics, and the selection pattern that gives rise to senescence, are not well known. To better understand this, it is necessary to quantify genetic variation in telomere length, and how such variation changes with age. Here, we analysed a longitudinal dataset (2,156 samples from 1,267 individuals across 15 years) from a wild, insular house sparrow (*Passer domesticus*) population with complete life-history and genetic relatedness data. Using a series of 'animal' models, we confirmed that telomere length (TL): (1) changes with age, reflecting senescence in this population. We then showed that (2) TL is repeatable (15.7%, 95% CrI: 10.2–21.0%) and heritable (14.0%, 95% CrI: 8.7–19.5%); and, for the first time in the wild, (3) that TL shows a genotype-by-age interaction, meaning that genotypes differ in their rate of TL change, where additive genetic variance increases at older ages. Our findings provide empirical evidence from a wild population that supports hypotheses explaining the evolution of senescence, and highlights the importance of telomere dynamics as a key biomarker of body physiology for the evolution of senescence.

Significance Statement

Telomere length and shortening are linked with survival, and are senescence biomarkers. Estimating heritable variation in telomere dynamics, and how this changes with age, allows us to better understand the evolution of senescence. Here, we investigated this using long-term data from a wild house sparrow population. We found that telomere length is heritable and thus has the potential to evolve. Our study provides the first empirical evidence concerning telomere length that supports evolutionary hypotheses of senescence – an increase in genetic variation of telomere length at older ages. Our study provides insights into the selection patterns that give rise to senescence, supports evolutionary predictions for a fitness-related trait, and encourages further investigation into telomere dynamics as a biomarker of senescence.

Main Text

Introduction

Telomeres are highly-conserved, repeating DNA sequences primarily capping the ends of chromosomes (1), and, e.g. by preventing end-to-end chromosome fusion (2), are important in maintaining DNA integrity. Telomeres protect coding DNA from erosion caused by the lagging strand of linear DNA not being fully replicated, i.e. the end-replication problem. Thus, in each cell replication cycle, telomeres shorten (2). Telomere shortening can also be induced by, e.g., stress exposure (3, 4), and be elongated by telomerase action (5), which is typically suppressed in adults to prevent unbounded growth (2). When telomeres shorten to a critical length, cell division ceases, and the cell enters a state of senescence (6).

The accumulation of senescent cells can negatively impact neighbouring cells, resulting in a decline in tissue function (7). As such, cell and organismal senescence is linked with telomere shortening. While the specific causal mechanism is still unclear (8), there is evidence that short telomeres, and/or telomere shortening, are linked to decreased survival in natural populations (9–12), and age-related disease and mortality in humans (6). Therefore, telomere length could reflect the intrinsic state of an individual by incorporating the physiological cost of stress-inducing activities such as reproduction (13). Consequently, telomere length can be under selection, and play a part in the evolution of senescence.

To confirm that telomere length could evolve, and to test the theories explaining the evolution of senescence on telomere length, one needs to demonstrate the presence of its genetic variance.

Estimates for the proportion of additive genetic variance (V_a) to total phenotypic variance (V_t) – the heritability – range from 0 to 1 among vertebrate studies (14, 15). This variation is partly driven by the choice of statistical methods, as commonly applied methods confound genetic and common environmental effects, resulting in inflated heritability estimates (14). Also, the majority of heritability estimates come from laboratory animals of controlled ages and environments, limiting our ability to deduce the roles of selection and evolution under natural conditions (16). Furthermore, under natural conditions, genotype-by-age interactions (G×A) are likely to occur when genotypes differ in their rate of senescence, resulting in an increase in V_a with age (17).

Such age-related changes in V_a can be indicative of selection patterns and evolutionary processes that give rise to senescence itself (16). Two non-mutually-exclusive evolutionary hypotheses explain the origin of senescence (18): (1) The mutation accumulation hypothesis posits that, due to extrinsic mortality risks, cohorts decline in number and reproductive potential as they age, weakening the selection pressure against deleterious mutations in later life, and allowing senescent phenotypes to persist (19). (2) The antagonistic pleiotropy hypothesis posits that, as population size is larger in younger age classes, pleiotropic mutations that provide benefits in early life but have damaging effects in late life would be selected for (20). Both hypotheses predict G×A, where selection weakens with age, leading to increasing V_a in senescing traits, while the antagonistic pleiotropy theory additionally predicts a negative genetic correlation between early life and late life trait values (16). Studies examining G×A in the wild have, however, provided mixed results – significant G×A in fitness-related traits has been found in some species (21–23) but not others (24, 25). Thus far, only two studies have tested for G×A in telomere length: one found significant G×A in telomere length in dairy cattle (*Bos taurus*, (26)), but the other, using a wild population of common terns (*Sterna hirundo*, (27)), did not. Here, we found G×A in telomere length in a wild, isolated house sparrow population (*Passer domesticus*), and demonstrated that telomere length senesces as predicted by evolutionary theory.

Results

We used longitudinal data (2,156 samples from 1,267 sparrows, 2000–2015), where the exact age of repeatedly sampled individuals is known (28). We verified that telomeres shortened as individuals aged, as telomere length (TL) was negatively correlated with within-individual age, but not across birds of different ages (Fig. S1, Table S1). TL did not differ between the sexes (Table S1). TL showed moderate repeatability and heritability (Model 6, individual repeatability = 15.7% (95% CrI: 10.2–21.0%), heritability = 14.0% (95% CrI: 8.7–19.5%), Table S2, Fig. 1). Little variation was explained by rearing parent identities, or by cohort, but capture year accounted for 11.9% (95% CrI 5.0–26.4%; Model 6, Table S2, Fig. 1) of the phenotypic variance in TL. The rates of telomere shortening with age differed among individuals, as there was statistically significant variance in their intercepts and slopes in our random regression model (Table 1). Across individuals, a longer TL was also associated with a slower rate of telomere shortening, indicated by a statistically significant positive covariance between the individual intercept and slope (less negative slope; Table 1).

Most importantly, we detected a G×A effect in telomere length, indicated from the random regression 'animal' model, where both TL and the rate of TL change had a statistically significant additive genetic component (Table 2). Longer telomeres were genetically correlated with slower telomere shortening, as we detected significant genetic covariance between the intercept and the slope (Table 2). Finally, the inter-age additive genetic matrix showed that V_a decreased up to age 3, and then increased at later ages (Fig. 2, Table S3).

Discussion

Here, for the first time in a wild population, we provide evidence for individuals differing in the rate of telomere shortening, and that this shortening has a genetic component signifying G×A. These

results support both the mutation accumulation and antagonistic pleiotropy hypotheses that explain the evolution of senescence.

We also showed, in our population, that TL undergoes senescence in adults, largely in line with reports in other natural systems (29–31). Beyond the rapid growth period during early life, telomeres in adults generally decline at a slower but steady rate, chiefly due to the accumulation of environment-induced damage and the general suppression of lengthening mechanisms (32). However, in our system, the TL for older birds was on average similar to that for younger ones, likely because old individuals with short telomeres had not survived and were thus not sampled, resulting in the levelling off of the between-individual relationship between age and TL. This selective disappearance, together with the senescence of TL, suggests that telomere dynamics could be linked to actuarial senescence in survival or lifespan, and could thus serve as a biomarker for senescence.

Adult TL in our population has the potential to undergo microevolution, provided there is selection. Our heritability of 14.0% is low compared to the global average among vertebrates (44.9%, (14)), and to some bird studies: 99% in zebra finches (*Taeniopygia guttata*, (33)), 81% in tree swallows (*Tachycineta bicolor*, (34)), 77% in jackdaws (*Corvus monedula*, (35)), 65% in common terns (*Sterna hirundo*, (27)), and 48% in great reed warblers (*Acrocephalus arundinaceus*, (36)), but is higher than others: 3.1–8.0% in Seychelles warblers (*Acrocephalus sechellensis*, (37)), and 3.8% in white-throated dippers (*Cinclus cinclus*, (38)). Such inconsistency among studies could have a biological explanation, for example being due to stronger selection pressure reducing genetic variation in our population. However, telomere length heritability estimates are also influenced by telomere assay, the statistical methods used to estimate heritability, and potentially age at sampling (14), which differed between these studies. During ageing, telomere length is expected to become less heritable as it becomes increasingly dependent on the environment – e.g. oxidative stress and various toxins can accelerate telomere attrition (32), and reduce the activity of telomerase, a major telomere lengthening mechanism (39). As such, in contrast to this study on adult telomere length, early-life telomere length (34–36) would be expected to exhibit higher heritability due to lower exposure to the environment and thus show lower variation (15).

Annual stochasticity, which encompasses environmental factors that could induce stress, explained a relatively large proportion of variance, of 11%. In contrast, rearing parent identities did not explain variation in TL, despite better parental care or foster parental quality being associated with longer offspring telomeres (40, 41). Our results suggest that, in our study population, parental effects on juvenile TL, if any, may not carry over into adulthood. Storage time also had a significant effect on TL, in line with previous experimental findings in this population (42).

To our knowledge, this is the first study to report G×A in telomere length in a natural population. We demonstrated that the rate of change in TL is partially genetically determined, which is expected, as telomere dynamics are complex and influenced by the combined action of many genes and cellular processes (43, 44). However, much remains unknown about telomere maintenance and repair mechanisms, such as the expression of telomerase, other than that it varies vastly across different taxa (45), or how increased antioxidant capacity reduces telomere loss (32). Our results emphasize the importance of examining both the genetic and environmental influences on these potential cellular pathways, and, on an evolutionary level, the importance of determining whether the rate of telomere shortening is genetically correlated with fitness, to quantify the selection acting on telomere dynamics.

Testing for G×A has also allowed us to study changes in genetic variation across ages. In our study population, V_{a} in TL increased from the age of 3 years onwards, in agreement with both the mutation accumulation and antagonistic pleiotropy theories of senescence (19, 20), which both

assume that selection pressures weaken at older ages, allowing suboptimal genotypes and thus greater genetic variation to remain in the population. We also discovered negative genetic covariance in TL between early and late age classes, suggesting evidence for antagonistic pleiotropy (16, 46) – genes promoting longer telomeres in early life also lead to shorter telomeres in late life. However, as these negative correlations were not observed throughout all early ages, this interpretation should be made with caution.

We detected a decrease in V_{a} from ages 0 to 3 years, contrary to an expected uniform increase in genetic variation in fitness-related traits undergoing senescence (17). There are two plausible explanations for this observed pattern. The first possibility is that certain genotypes lead to telomere lengthening, and that opposing aging trajectories in TL intersect in middle life, causing higher genetic variance in both early and late life (17). However, we consider this explanation unlikely, as telomere lengthening currently lacks support in birds (but see (47), and e.g. (48, 49) in mammals). Furthermore, lengthening could easily be masked by methodological effects such as measurement error (49), leukocyte composition changes, and storage time effects, which significantly influenced TL in our dataset. It is much more likely that the decrease in V_a during early life pertained to mortality risks. In the Lundy house sparrows, individuals attain their highest reproductive effort at around three to five years of age (Fig. S2). As reproduction could be energetically costly, it could be that intrinsic mortality risks are at their highest where reproductive effort peaks, eliminating individuals of low quality from those age classes and thereby reducing individual and genetic variation in TL. This idea is corroborated by a study on age-related mortality in the same population, where mortality increased after a trough at around two years of age (50). To confirm whether the observed G×A pattern is associated with varying mortality with age, and to better understand how selection shapes senescence in telomere length, a next step would be to quantify selection pressures with age, for example by modelling genetic correlations between age-specific telomere length and fitness.

Materials and Methods

Study population and data collection

The house sparrow (*Passer domesticus*) is a gregarious and socially monogamous passerine that readily uses nestboxes, and is sedentary in nature with limited movement (51). In this study, we collected telomere, life-history and pedigree data from a wild nestbox-breeding population of house sparrows on Lundy Island (51°10'N, 4°40'W), 19 km off the coast of Devon, United Kingdom. We systematically monitored this population starting from 2000. Owing to the small size of the island and its geographical isolation limiting immigration and emigration (28), we were able to tag and identify >99% of all sparrows hatched on Lundy since 2000 with a uniquely numbered metal ring from the British Trust for Ornithology and a unique combination of three colour rings. Every year, we recorded all nestbox breeding data including observed parent identities, offspring identities and hatch dates, allowing the exact age of each bird to be recorded. A small minority of birds fledged from inaccessible nests, and we captured these with mist nets, both during the breeding season immediately after they fledged (April to August) and during the following annual winter census visit (November to December). We assumed these birds hatched during the breeding season of that year.

To quantify telomere length and assign genetic parentage, we collected blood samples repeatedly from individuals, typically at two and 12 days of age, during their first winter, and on every subsequent capture. Previous analysis of this population suggests that all birds were equally likely to be caught and sampled (52). We stored blood samples in absolute ethanol at room temperature until DNA extraction. In addition, to distinguish the effects of the genetic parents, the environment during incubation, and the environment post-hatching, on average 39% of chicks were cross-fostered at two or three days of age during every breeding season (53). All animal procedures were approved by the British Trust for Ornithology and the UK Home Office.

Telomere extraction and assay
We measured telomere length (TL) using blood samples collected from sparrows after fledging, between 2000 and 2015. We extracted DNA using an ammonium acetate extraction method, following (54). Extracted DNA was stored in TE buffer (10 mM Tris, 0.1 mM EDTA) at -20°C until telomere analysis. Prior to telomere assays, DNA concentration was measured using a Nanodrop 8000 Spectrophotometer (Thermo Fisher) and normalized to 20–30 ng/µl. Following normalization, we employed a monochrome multiplex quantitative polymerase chain reaction (MMqPCR) method to quantify TL (55) (For details, see Supporting Information).

Reactions were run using two machines, a QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific, five plates) and a StepOnePlus (Applied Biosystems, 77 plates), but machine identity did not have an effect on the final T/S ratios (42). Plates were run by two technicians (MEM ran 52 plates and NdR ran 30 plates). The final dataset consisted of 2,156 telomere length measurements from 1,267 birds, 489 of which have at least two telomere length observations. Further summaries of the telomere dataset are provided in Tables S4 and S5.

Genetic pedigree construction
We used up to 23 house sparrow microsatellite markers previously published (56) to construct a genetic pedigree for individuals born 1995–2019, using Cervus 3.0 (57). In brief, we first ran an identity analysis to resolve potential field sampling and lab errors, then ran a maternal analysis to confirm the genetic mother, and finally, a biparental analysis to assign the genetic father (for details, see supplementary information in (58)). We then pruned the pedigree to include only informative individuals, i.e. individuals with telomere length measurements and those linking these individuals. The pruned pedigree consisted of 1,362 birds, with 1,238 assigned maternities and 1,237 paternities, and a maximum pedigree depth of 16 generations (Fig. S3). Statistics for both the full and pruned pedigrees were calculated using the R package *pedantics* 1.7 (Morrissey & Wilson, 2010) and summarized in Table S6.

Statistical analysis
All analyses were carried out in R 4.0.3 (60). Regression models were built using the Bayesian package *MCMCglmm* 2.29 (61). For each model, we adjusted the number of iterations, burn-in, and thinning interval, such that convergence was reached based on the following criteria: visual inspection of posterior trace plots showed no distinguishable trend, autocorrelation was lower than 0.1, the effective sample size was greater than 1000, and that no more than one MCMC chain failed the Heidelberger and Welch's and the Geweke convergence tests.

(1) Age-dependent changes in telomere length
To first verify that TL varies with age, we built a linear mixed model (LMM, Model 1), where T/S ratio was the response variable assuming a Gaussian residual distribution. Log-transforming TL did not provide a better model fit. To examine individual senescence patterns, we separated within-individual and between-individual effects by fitting both the age mean-centred within each individual (WiAge, in years) and the mean age of each individual (BtAge, in years) as explanatory variables (62). We also tested for a non-linear relationship by fitting second-order terms for both WiAge and BtAge. Fitting age mean-centred over the whole population (McAge, in years), or age as a factor did not provide a better model fit. To test for differences in TL between males and females, we fitted sex as a two-level fixed factor. As it was experimentally proven that TL decreases with sample storage time in our dataset (42), we fitted the duration for which the blood sample was stored before DNA extraction (Blood Age, in years), the duration for which the extracted DNA was stored before telomere assay (DNA Age, in years), and their squared terms. As TL differed between the two technicians (Wilcoxon rank sum test: $W = 300623$, $p<0.001$), we also added technician ID as a two-level fixed factor. Finally, as random variables we fitted individual bird ID to account for variation in TL among birds, as well as plate ID and row ID to account for technical variance among qPCR plates and among row positions on each plate (63). We used default (flat improper, weakly informative) priors for fixed effects, and uninformative

inverse-Wishart priors (V = 1, nu = 0.002) for random effects. The model remained robust when another relevant prior (parameter expanded prior: V = 1, nu = 0.002, alpha.mu = 0, alpha.V = 1000) was used (Table S7).

The TL–age relationship was linear in our population and sex had no effect on TL (see Results), and hence we removed the quadratic terms of WiAge, BtAge, and sex from the fixed effects structure in subsequent analysis. The removal of these terms did not impair model fit (ΔDIC = -4.515). As a significant difference between the within- and between-individual slopes could lead to a biased estimation of the individual variances in the random effects structure (64), we tested for this difference by further fitting an LMM (Model 2), where WiAge was replaced with untransformed age (in years):

(i) $$TL \sim Untransformed\ age + BtAge + BloodAge + BloodAge^2 + DNAAge + DNAAge^2 + TechnicianID + (1|BirdID) + (1|Plate) + (1|Row)$$

In this model, the untransformed age effect represents the within-individual slope, while the BtAge effect represents the difference between the within- and between-individual slope (62). The two slopes were statistically significantly different from each other (posterior mode for BtAge = 0.08, 95% CrI = 0.04–0.11).

(2) Telomere length repeatability and heritability

To estimate the additive genetic (V_{a}) and permanent environmental variance (V_{pe}) in TL, we expanded Model 1 into a series of 'animal' models with sequentially increasing random variables. In Model 3, we fitted an individual 'animal' term linked to the pruned pedigree, in addition to the individual 'bird ID' term, allowing the separation of individual variance into genetic and permanent environmental components. In Models 4 and 5, we added the identity of the rearing mother and father, respectively, to estimate the variance due to non-genetic parental effects during rearing. In Model 6, we added the year of capture to account for potential yearly environmental stress effects on TL. Finally, in Model 7, we added the year in which the individual was born (cohort) to estimate the effect of the hatching year. For each model, we calculated individual repeatability as (V_{a} + V_{pe}) / V_{t}, and heritability as V_{a} / V_{t}, where V_{t} is the sum of all variance components and residual variance, except those of plate ID and row ID, as these technical variances are biologically irrelevant. We further calculated the variance explained by the fixed effects WiAge and BtAge, as not including fixed effects variances in V_{t} could lead to upward bias in repeatability and heritability estimates (65). However, both fixed effects explained minimal variance (< 0.002), and therefore we did not include them in the final calculation of V_{t}. In all 'animal' models, we used default priors for fixed effects, parameter-expanded priors (V = 1, nu = 1, alpha.mu = 0, alpha.V = 1000) for random effects, as they improve mixing at the parameter space boundary (66), and models using inverse-Wishart priors did not converge. We used inverse-Wishart priors for residuals.

(3) Individual variation in the rate of telomere shortening

We tested whether individuals differ in their rates of telomere shortening (individual-by-age interaction, or I×A), as such variation would allow scope for G×A. To test for I×A, we fitted a random regression model (RRM), with TL as the response variable. For the fixed effects structure, we fitted McAge and retained all storage variables and technician ID from the previous models. For the random effects structure, we modelled individual variation in TL as a function of age, in addition to effects of the year of capture, plate ID and row ID. We excluded identities of the rearing parents and cohort in the random effect structure, as these variables did not explain any biologically meaningful variance (see results). The final model equation for the RRM is thus:

(ii) $$TL = \mu + McAge + BloodAge + BloodAge^2 + DNAAge + DNAAge^2 + TechnicianID + f(ID, age^*) + Capture\ year + Plate + Row + \varepsilon$$

where $f(ID, age^*)$ is the random regression function for individuals. For this random effect, we used Legendre polynomials following (23) and (67), where age is rescaled to a range of -1 to 1 (from 0 to 7) by:

(iii) $$age_i^* = -1 + \frac{2}{age_{max} - age_{min}} (age_i - age_{min})$$

where age_i^* is the rescaled age, age_i is the original age, age_{max} is the maximum age recorded in the whole dataset, and age_{min} is the minimum age recorded in the whole dataset. While the choice of the class of orthogonal polynomials does not affect estimation of inter-age covariances over the age range in which the data were collected, it would affect extrapolation outside of this range (67). As we only found a linear TL–age relationship within individuals, we only fitted the first two Legendre polynomials:

$$\text{(iv)}\ \varphi_0 = \frac{1}{\sqrt{2}}$$

$$\text{(v)}\ \varphi_1 = \sqrt{\frac{3}{2}}x$$

We fitted a homogenous residual structure since the RRM with a heterogeneous residual structure, where one residual variance was estimated for each age, did not converge. We used inverse-Wishart priors to estimate both random and residual structures.

(4) Changes in additive genetic variance in relative telomere length

To assess whether the rate of telomere shortening had a genetic basis, we built a random regression animal model (RRAM) from the RRM above, where we partitioned the individual variation in the TL–age slope into genetic and permanent environmental components by fitting an 'animal' random effect term linked to the genetic pedigree. We retained the same fixed effects structure, additional random variables, and residual structure as for the RRM, above. Thus, the model equation was:

$$\text{(vi)}\ TL = \mu + McAge + BloodAge + BloodAge^2 + DNAAge + DNAAge^2 + TechnicianID + f(a, age^*) + f(pe, age^*) + Capture\ year + Plate + Row + \varepsilon$$

where $f(a, age^*)$ represents the random regression function for the additive genetic effect and $f(pe, age^*)$ that of the permanent environmental effect. As the RRAM experienced difficulty in allocating individual variance to the 'animal' and BirdID terms, we ran a second RRAM including the 'animal' term only to confirm our results. This second RRAM returned similar additive genetic variance and covariance estimates (Table S8). We further confirmed that the genetic variance was statistically significant, as model fit was improved by including the animal term (ΔDIC = -32.34). To examine the changes in V_a with age, we transformed the estimates of the additive genetic coefficients from the RRAM by

$$\textit{(vii)} \qquad G = \Phi C \Phi^T$$

where G is the inter-age additive genetic variance-covariance matrix, C is the RRAM coefficient matrix, and Φ is a matrix defined such that $\Phi_{ij} = \varphi_0(age*_i)$ (67).

Data availability

Datasets and R code used in this study will be available on FigShare or other public repositories upon acceptance of the manuscript.

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Figures and Tables

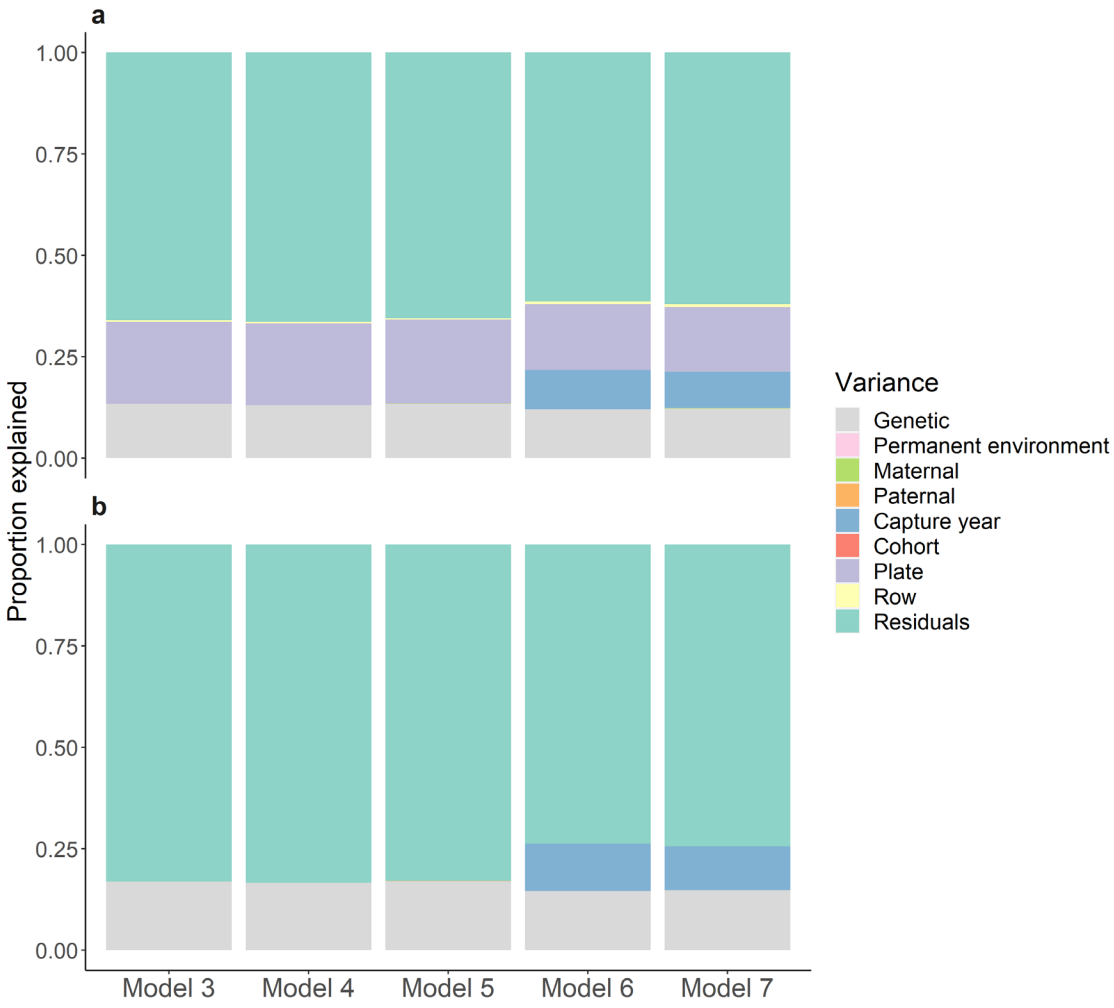

Figure 1. Variance components from a series of 'animal' models (Models 3–7) to estimate sources of variation in telomere length in the Lundy house sparrow population sampled in 2000–2015. (a) the proportions of all fitted random variables, (b) proportions of biologically relevant random variables only, i.e. excluding plate and row variances.

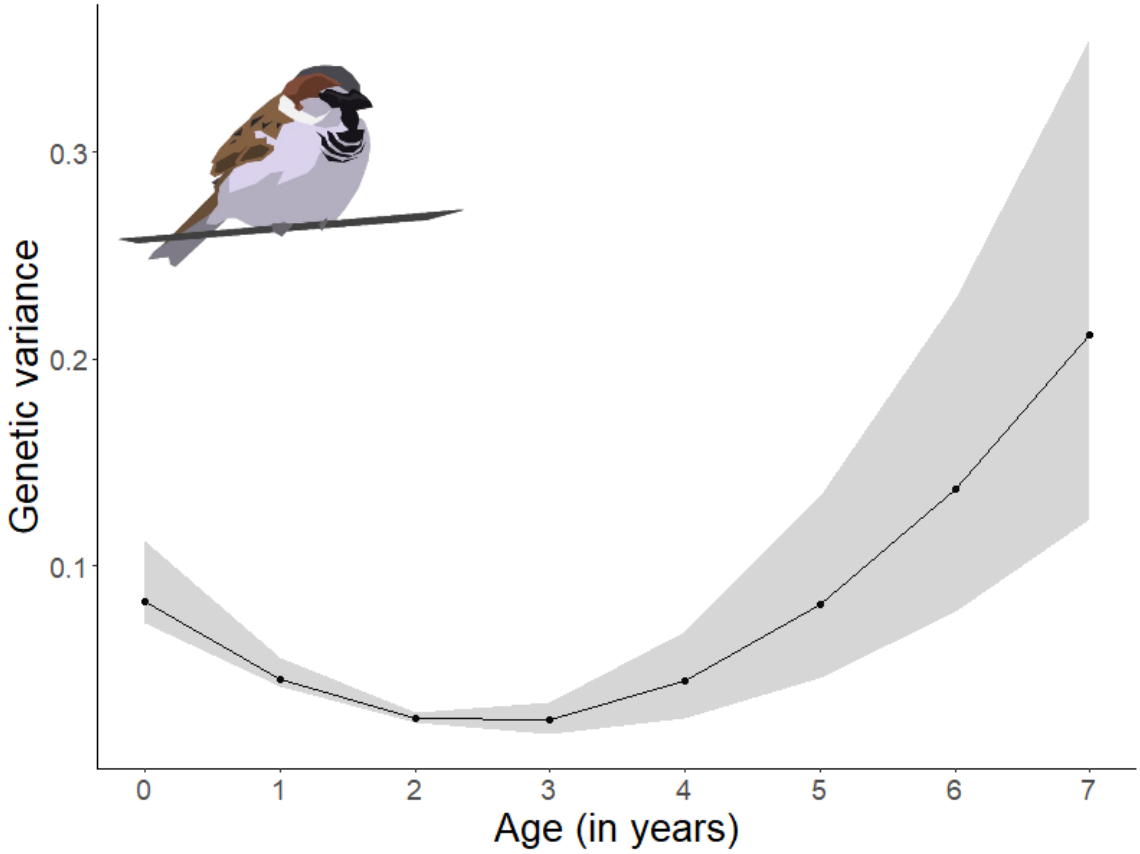

Figure 2. The quadratic relationship between additive genetic variance of telomere length and age in Lundy house sparrows sampled in 2000–2015. Black dots represent point estimates of additive genetic variance for each age class (0–7), and the shaded area around each dot represents the 95% CrI of the respective point estimate.

Table 1. Summary of the random regression model (RRM) testing for individual variation in the mean telomere length (TL), and rate of TL change with age, among Lundy house sparrows sampled in 2000–2015. Statistically significant estimates are in bold. Post. mode = posterior mode, 95% CrI = 95% credible interval; pMCMC = MCMC p-value. McAge = population mean-centred age; Blood Age = storage time as blood sample (in years); DNA Age = storage time as DNA sample (in years); Technician (N = 2; contrast = A); BirdID = unique individual identifier; Year = Year of capture; Plate = qPCR plate ID; Row = Row ID on qPCR plate; Units = residuals.

	Post. mode	*95% CrI*	*Effective sample size*	*pMCMC*
Fixed effects				
(Intercept)	**1.503**	**1.275 – 1.723**	**180000**	**<0.0001**
McAge	-0.003	-0.020 – 0.017	180310	0.861
Blood Age	**-0.092**	**-0.130 – -0.050**	**180000**	**<0.0001**
Blood Age^2	**0.003**	**0.001 – 0.006**	**180000**	**0.009**
DNA Age	0.008	-0.042 – 0.055	179127	0.801
DNA Age^2	**-0.004**	**-0.009 – -0.000**	**177187**	**0.033**
Technician (B)	0.069	-0.065 – 0.214	180000	0.299
Random effects				
BirdID				
Intercept	0.060	0.042 – 0.088	32105	
Slope	0.077	0.052 – 0.122	28380	
Intercept: Slope	**0.030**	**0.010 – 0.060**	**26363**	
Year	0.025	0.011 – 0.071	178679	
Plate	0.051	0.034 – 0.079	178351	
Row	0.001	0.000 – 0.008	181322	
Units	0.160	0.147 – 0.173	144926	

Table 2. Summary of the random regression 'animal' model (RRAM) testing for additive genetic ('Animal' term) and permanent environmental ('Bird ID' term) variation in the mean telomere length (TL), and rate of TL change with age, among the Lundy house sparrows sampled in 2000–2015. Statistically significant estimates are in bold. Post. mode = posterior mode, 95% CrI = 95% credible interval; pMCMC = MCMC p-value. McAge = population mean-centred age; Blood Age = storage time as blood sample (in years); DNA Age = storage time as DNA sample (in years); Technician (N=2; contrast = A); Animal = genetic variances and covariances; BirdID = permanent environmental variances and covariances; Year = Year of capture; Plate = qPCR plate ID; Row = Row ID on qPCR plate; Units = residuals.

	Post. mode	*95% CrI*	*Effective sample size*	*pMCMC*
Fixed effects				
(Intercept)	**1.546**	**1.116 – 2.017**	**45000**	**<0.0001**
McAge	-0.004	-0.039 – 0.036	45000	0.926
BloodAge	**-0.088**	**-0.139 – -0.035**	**45644**	**0.001**
BloodAge^2	**0.003**	**0.000 – 0.006**	**45000**	**0.019**
DNAAge	0.010	-0.046 – 0.070	45000	0.696
DNAAge^2	**-0.005**	**-0.010 – -0.001**	**44626**	**0.019**
Technician (B)	0.058	-0.092 – 0.213	49403	0.452
Random effects				
Animal				
Intercept	0.066	0.042 – 0.095	39704	
Slope	0.076	0.051 – 0.124	38429	
Intercept:Slope	**0.037**	**0.014 – 0.070**	**36773**	
BirdID				
Intercept	0.057	0.039 – 0.085	41108	
Slope	0.074	0.050 – 0.118	40165	
Intercept:Slope	**0.039**	**0.017 – 0.068**	**39926**	
Year	0.086	0.046 – 0.223	45000	
Plate	0.063	0.042 – 0.092	45000	
Row	0.108	0.042 – 0.375	45000	
Units	0.146	0.136 – 0.160	45804	

Supporting Information for

Heritability and age-dependent changes in genetic variation of telomere length in a wild house sparrow population

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Supporting Information Text

Telomere length quantification using monochrome multiplex quantitative PCR (MMqPCR). In this study we measured telomere length in house sparrow blood samples using MMqPCR. In brief, qPCR measures signals of telomeres (T) and those of a single-copy reference gene (S), and quantifies telomere length (TL) as the T/S ratio. Unlike conventional singleplex qPCR, where T and S amplification are conducted in separate wells (1), MMqPCR allows T and S signals to be obtained from the same reaction within a single well, thus eliminating error due to differences in the amount of DNA pipetted (2). Multiplexing is achieved by using specially designed primers that significantly raise the melting temperature of the S sequence, and by employing a special temperature profile: During earlier, low temperature cycles, cycle threshold (Ct) values of the more abundant telomeres are obtained, when the S signal is still at baseline; during later cycles, the temperature is raised well above the melting temperature of the telomeric sequences, such that Ct values of the S sequence can be obtained while the T signal goes to baseline. We used the primers telg and telc to prime telomere sequences (2), and GAPDH, primed by GAPDH-F (5'-CGGCGGCGGGCGGCGCGGGCTGGGCGGAGC-CAGCCAAGTACGATGACAT - 3') and GAPDH-R (5' – GCCCGGCCCGCCGCGCC-CGTCCCGCCGCCATCAGCAGCAGCCTTCA - 3') as the single-copy reference gene. The GC-clamps in GAPDH-F and GAPDH-R at the 5' end raise the melting temperature of the S sequence to achieve multiplexing. We loaded each well with 1.5 µl normalized DNA, 10 µl SYBR Select Master Mix (Applied Biosystems) and each of the four primers at 0.9 µM, totaling 20 µl of reaction mixture. We prepared additionally a series of standard reaction mixtures on each plate using three samples at 0.3125, 1.25, 5, 20 and 80 ng/µl, as well as two control wells where DNA was absent. The standard samples allowed the construction of a standard curve to calculate T and S product contents in the reaction mixtures. We ran all qPCR reactions in duplicate in adjacent wells, with the following steps: 95°C for 15 min; 2 cycles of 94°C for 15s and 49°C for 15s; 32 cycles of 94°C for 15s, 62°C for 10s and 74°C for 15s with signal acquisition, 84°C for 10s and 86°C for 15s with signal acquisition.

Following MMqPCR, we derived T/S ratios from each reaction using a visual thresholding method: First, we determined background T and S florescent signals as the average signal values of the earliest cycles before the exponential phase, and subtracted these background values from the raw signals. Second, we plotted log-transformed signal values against cycle number, and visually determined the threshold signal value for both T and S products as the value at the mid-point of the exponential phase. We then calculated T and S Ct values as the expected number of cycles required to reach the threshold. T- and S-values, i.e. the quantity of T and S products in each well, were then calculated from the slope and intercept of the standard curve, and the T/S ratios were finally calculated as T-value / S-value. We removed samples with Ct values >25 as they were deemed outliers and unreliable. We further calculated the relative difference in T/S ratio between duplicates and removed samples with relative difference >0.2, and averaged the T/S ratios of the remaining duplicates as the final measure of TL for each sample.

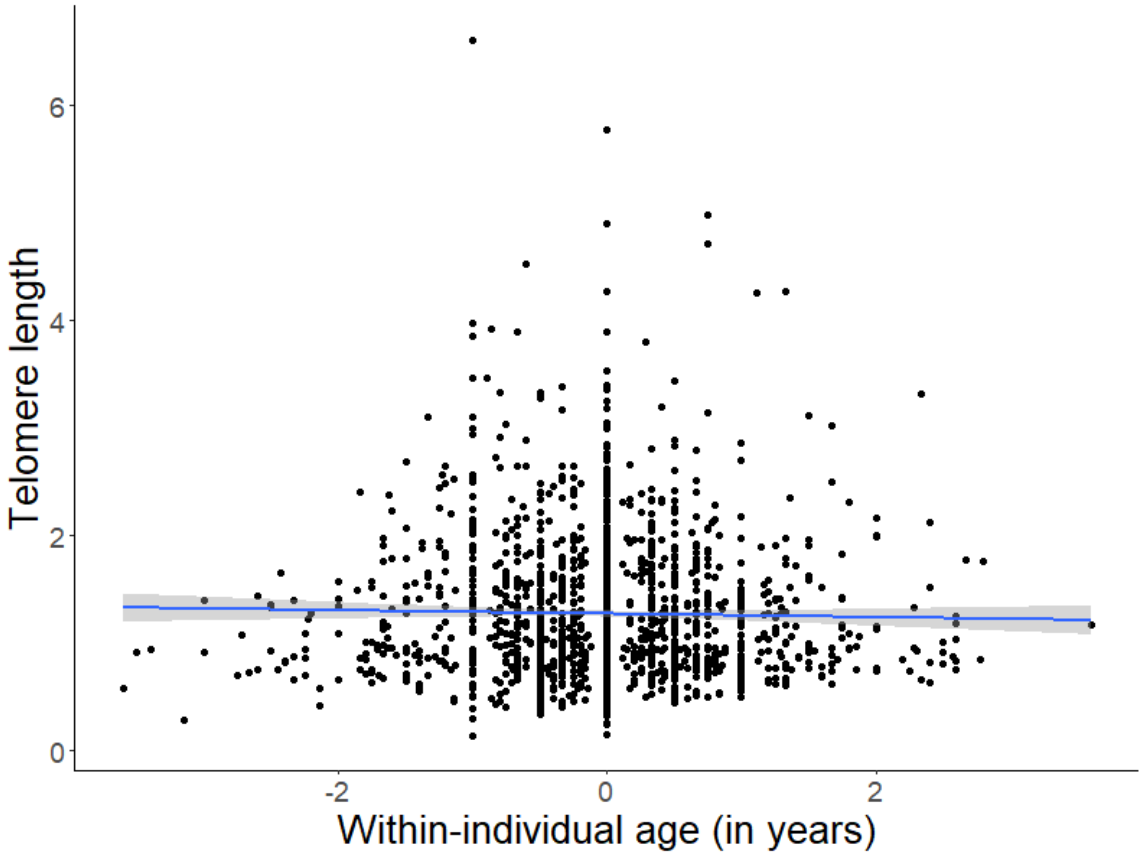

Fig. S1. Linear relationship between telomere length and within-individual mean-centred age estimated from the Lundy house sparrows sampled in 2000-2015. The blue line indicates the predicted relationship, while the shaded area represents the 95% CrI.

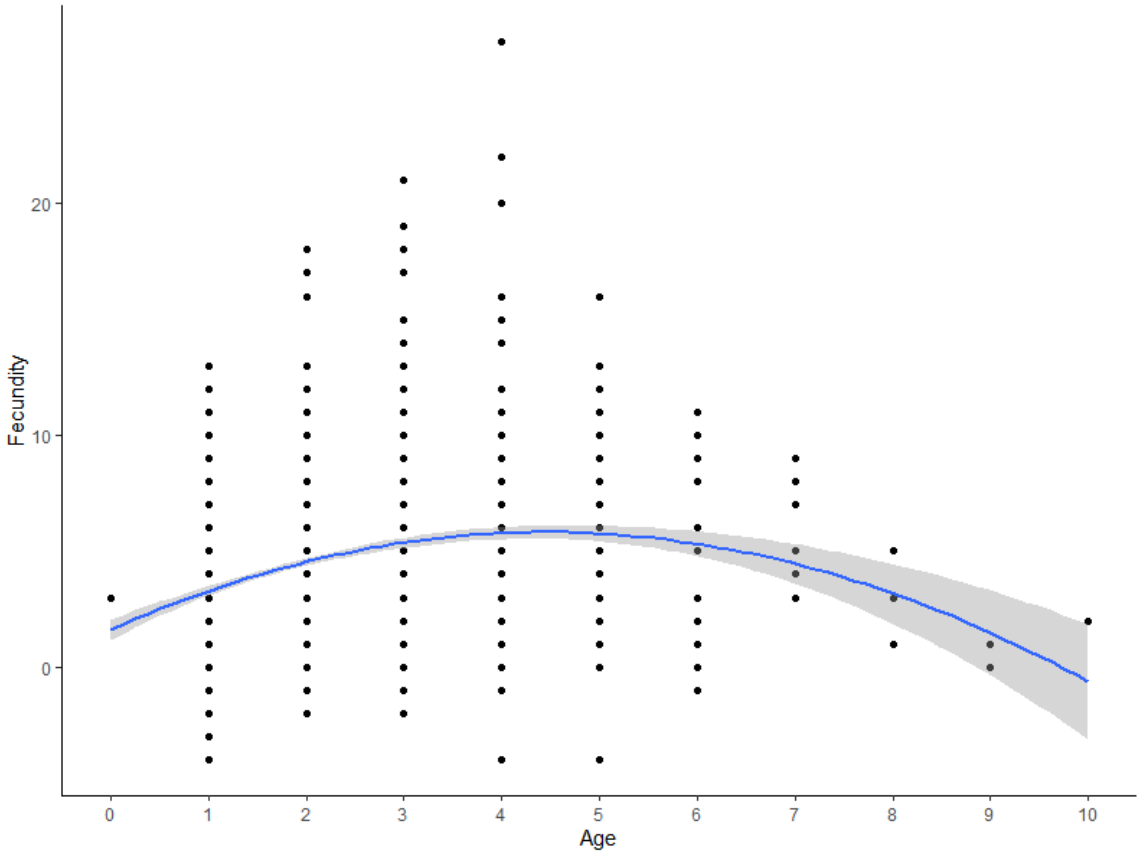

Fig. S2. Annual fecundity across age in the Lundy house sparrows. Blue line represents predicted the fecundity–age relationship, and shaded area represents 95% confidence intervals.

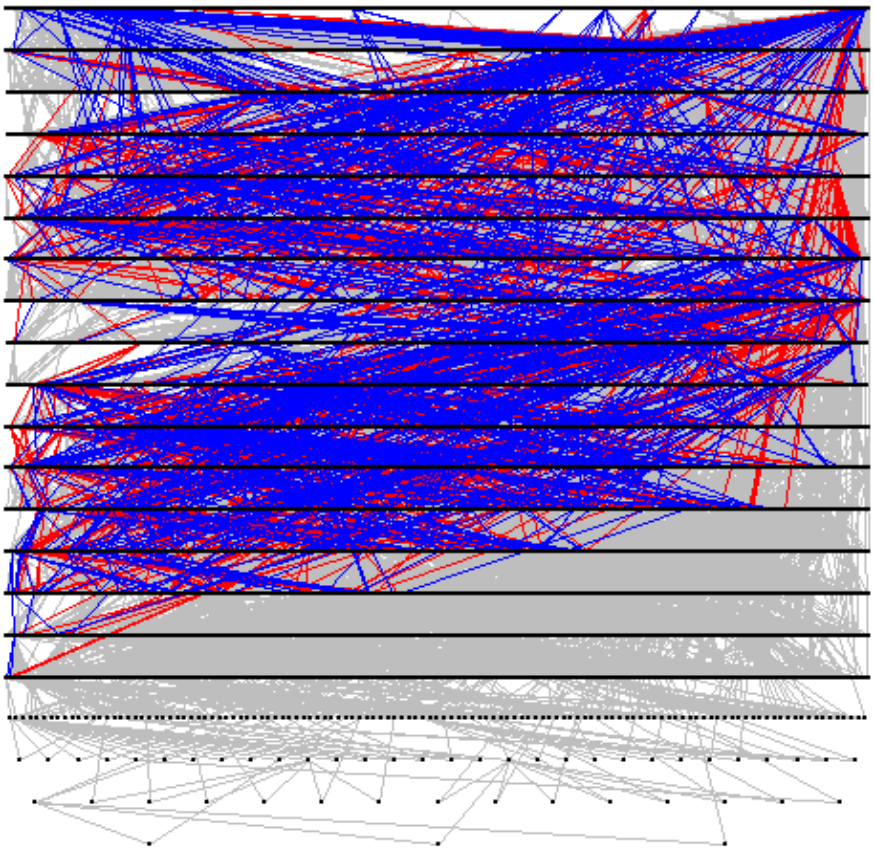

Fig. S3. The pruned pedigree of the Lundy House Sparrows used to estimate heritability of telomere length and rate of telomere shortening in the study. Each dot represents one individual, red lines represent maternities, blue lines represent paternities, and grey lines represent links with non-phenotyped but informative individuals.

Table S1. Summary of the full linear mixed model (LMM) testing for the telomere length–age relationship in the Lundy house sparrows sampled from 2000-2015, using inverse-Wishart priors. Statistically significant estimates are in bold. Post. Mode = posterior mode, 95% CrI = 95% credible interval; pMCMC = MCMC p-value. WiAge = Within-individual age (in years); BtAge = between-individual age (in years); Blood Age = storage time as blood sample (in years); DNA Age = storage time as DNA sample (in years); Technician (N=2; contrast = A); BirdID = unique individual identifier; Plate = qPCR plate ID; Row = Row ID on qPCR plate; Units = residuals.

	Post. mode	*95% CrI*	*Effective sample size*	*pMCMC*
Fixed effects				
(Intercept)	**1.365**	**1.236 – 1.541**	**9553**	**<0.0001**
WiAge	**-0.054**	**-0.086 – -0.029**	**9000**	**<0.0001**
$WiAge^2$	-0.010	-0.028 – 0.008	9000	0.299
BtAge	0.040	-0.010 – 0.091	9000	0.128
$BtAge^2$	-0.005	-0.016 – 0.008	9000	0.538
Sex	0.008	-0.031 – 0.057	9000	0.615
Blood Age	**-0.072**	**-0.103 – -0.047**	**9000**	**<0.0001**
Blood Age^2	**0.002**	**0.001 – 0.004**	**9000**	**0.007**
DNA Age	0.010	-0.027 – 0.044	9000	0.668
DNA Age^2	**-0.005**	**-0.007 – -0.001**	**9000**	**0.011**
Technician(B)	**0.154**	**0.024 – 0.324**	**9000**	**0.017**
Random effects				
BirdID	0.034	0.020 – 0.048	8606	
Plate	0.072	0.046 – 0.105	9000	
Row	0.001	0.000 – 0.007	9000	
Units	0.193	0.178 – 0.211	9000	

Table S2. Random effect structures, individual repeatability (R), heritability (h^2) and DICs from a series of 'animal' models estimating sources of variation in telomere length in the Lundy house sparrows sampled in 2000-2015. We report here the posterior modes and 95% credible intervals of estimates. Animal = Additive genetic variance estimated from genetic pedigree; Bird ID = permanent environmental variance; Rearing Mum ID = identity of rearing mother; Rearing Dad ID = identity of rearing father; Year = Year of sampling; Cohort = Year when an individual was born; Plate ID = qPCR plate identity; Row ID = row where sample was located on the qPCR plate. Plate and row variance were included in the models but excluded when calculating R and h^2.

Model	*Animal*	*Bird ID*	*Rearing Mum ID*	*Rearing Dad ID*	*Year*	*Cohort*	*Plate ID*	*Row ID*	*Residual*	*R*	*h^2*	*DIC*
3	0.039 (0.024 – 0.054)	0.000 (0.000 – 0.013)	/	/	/	/	0.065 (0.042 – 0.096)	0.001 (0.000 – 0.008)	0.190 (0.177 – 0.206)	0.176 (0.129 – 0.240)	0.164 (0.111 – 0.224)	2856
4	0.037 (0.024 – 0.054)	0.000 (0.000 – 0.013)	0.000 (0.000 – 0.002)	/	/	/	0.063 (0.042 – 0.096)	0.001 (0.000 – 0.008)	0.192 (0.176 – 0.205)	0.182 (0.128 – 0.234)	0.165 (0.109 – 0.223)	2858
5	0.039 (0.024 – 0.054)	0.000 (0.000 – 0.013)	0.000 (0.000 – 0.002)	0.000 (0.000 – 0.002)	/	/	0.064 (0.042 – 0.097)	0.001 (0.000 – 0.008)	0.192 (0.176 – 0.206)	0.177 (0.128 – 0.239)	0.165 (0.108 – 0.221)	2859
6	0.035 (0.022 – 0.050)	0.000 (0.000 – 0.012)	0.000 (0.000 – 0.002)	0.000 (0.000 – 0.002)	0.027 (0.010 – 0.076)	/	0.048 (0.033 – 0.075)	0.002 (0.000 – 0.010)	0.177 (0.163 – 0.190)	0.157 (0.102 – 0.210)	0.140 (0.087 – 0.195)	2703
7	0.036 (0.022 – 0.049)	0.000 (0.000 – 0.012)	0.000 (0.000 – 0.002)	0.000 (0.000 – 0.002)	0.027 (0.010 – 0.076)	0.000 (0.000 – 0.003)	0.048 (0.033 – 0.076)	0.001 (0.000 – 0.010)	0.175 (0.163 – 0.190)	0.149 (0.100 – 0.208)	0.137 (0.087 – 0.194)	2704

Table S3. Inter-age variance-covariance matrix showing the age-related structure of additive genetic variance in telomere length in the Lundy house sparrows sampled in 2000-2015. Estimates (and 95% CrIs) for a total of eight age classes (Age 0 to Age 7) were obtained from back-transformation of random regression 'animal' model (RRAM) coefficients. Within age-class variances are shown on the diagonal while inter-age-class covariances are shown on the off-diagonal. Significant covariances are in bold.

	Age 0	*Age 1*	*Age 2*	*Age 3*	*Age 4*	*Age 5*	*Age 6*	*Age 7*
Age 0	0.083 (0.072 – 0.112)							
Age 1	**0.060 (0.054 – 0.076)**	0.045 (0.042 – 0.056)						
Age 2	**0.036 (0.036 – 0.041)**	**0.031 (0.030 – 0.035)**	0.026 (0.024 – 0.03)					
Age 3	**0.012 (0.005 – 0.018)**	**0.017 (0.014 – 0.018)**	**0.021 (0.019 – 0.024)**	0.026 (0.019 – 0.034)				
Age 4	**-0.011 (-0.031 – -0.001)**	0.003 (-0.006 – 0.006)	**0.017 (0.013 – 0.019)**	**0.030 (0.020 – 0.044)**	0.044 (0.026 – 0.068)			
Age 5	**-0.035 (-0.067 – -0.019)**	**-0.011 (-0.027 – -0.006)**	**0.012 (0.007 – 0.013)**	**0.035 (0.020 – 0.053)**	**0.058 (0.033 – 0.093)**	0.081 (0.046 – 0.133)		
Age 6	**-0.058 (-0.103 – -0.037)**	**-0.026 (-0.048 – -0.018)**	**0.007 (0.001 – 0.008)**	**0.040 (0.021 – 0.063)**	**0.072 (0.040 – 0.118)**	**0.105 (0.059 – 0.173)**	0.137 (0.078 – 0.229)	
Age 7	**-0.082 (-0.139 – -0.055)**	**-0.040 (-0.068 – -0.030)**	0.002 (-0.004 – 0.002)	**0.044 (0.021 – 0.073)**	**0.086 (0.047 – 0.143)**	**0.127 (0.072 – 0.214)**	**0.170 (0.097 – 0.284)**	0.212 (0.123 – 0.354)

Table S4. Summary of the number of individuals with various numbers of samples in the Lundy house sparrow telomere dataset collected in 2000-2015

Number of samples	*Number of individuals*
1	778
2	262
3	124
4	59
5	25
6	15
7	2
8	1
9	1

Table S5. Summary of the number of birds and samples across age classes in the Lundy house sparrow telomere dataset collected in 2000-2015

Age in years	*Number of birds*	*Number of samples*
0	750	875
1	534	668
2	248	298
3	144	175
4	64	78
5	35	40
6	15	15
7	5	7

Table S6. Summary of the full and pruned genetic pedigree of the Lundy house sparrows used in the telomere length analysis

Statistic	*Full pedigree*	*Pruned pedigree*
Records	10655	1362
Maternities	8823	1238
Paternities	8951	1237
Full sibs	41647	1355
Maternal sibs	118459	3628
Maternal half sibs	76812	2273
Paternal sibs	132081	3740
Paternal half sibs	90434	2385
Maternal grandmothers	7933	1120
Maternal grandfathers	8130	1131
Paternal grandmothers	7862	1083
Paternal grandfathers	7887	1082
Maximum pedigree depth	20	16
Founders	1636	111
Mean maternal sibship size	13.74	3.71
Mean paternal sibship size	13.07	3.77
Non-zero F	6054	829
F > 0.125	552	60
Mean pairwise relatedness	0.04	0.06
Pairwise relatedness ≥ 0.125	0.10	0.15
Pairwise relatedness ≥ 0.25	0.01	0.02
Pairwise relatedness ≥ 0.5	0.001	0.004

Table S7. Summary of the full linear mixed model (LMM) testing for the telomere length–age relationship in the Lundy house sparrows sampled from 2000-2015, using parameter-expanded priors. Statistically significant estimates are in bold. Post. Mode = posterior mode, 95% CrI = 95% credible interval; pMCMC = MCMC p-value. WiAge = Within-individual age (in years); BtAge = between-individual age (in years); Blood Age = storage time as blood sample (in years); DNA Age = storage time as DNA sample (in years); Technician (N=2; contrast = A); BirdID = unique individual identifier; Plate = qPCR plate ID; Row = Row ID on qPCR plate; Units = residuals.

	Post. mode	*95% CrI*	*Effective sample size*	*pMCMC*
Fixed effects				
(Intercept)	**1.382**	**1.234 – 1.542**	**9000**	**<0.0001**
WiAge	**-0.052**	**-0.084 – -0.027**	**9000**	**<0.001**
$WiAge^2$	-0.007	-0.028 – 0.009	9000	0.323
BtAge	0.037	-0.014 – 0.085	9000	0.128
$BtAge^2$	-0.005	-0.016 – 0.008	9097	0.542
Sex	0.014	-0.033 – 0.058	9000	0.601
Blood Age	**-0.078**	**-0.105 – -0.048**	**8525**	**<0.0001**
$Blood\ Age^2$	**0.002**	**0.001 – 0.004**	**9122**	**0.010**
DNA Age	0.003	-0.027 – 0.042	9000	0.663
$DNA\ Age^2$	**-0.004**	**-0.007 – -0.001**	**8953**	**0.011**
Technician(B)	**0.177**	**0.026 – 0.325**	**8072**	**0.016**
Random effects				
BirdID	0.033	0.021 – 0.049	9000	
Plate	0.072	0.047 – 0.108	9000	
Row	0.001	0.000 – 0.008	9000	
Units	0.195	0.178 – 0.210	9405	

Table S8. Summary of the random regression 'animal' model (RRAM) testing for only additive genetic ('Animal' term) variation in the mean telomere length (TL), and rate of TL change with age, among the Lundy house sparrows sampled in 2000-2015. Statistically significant estimates are in bold. Post. mode = posterior mode, 95% CrI = 95% credible interval; pMCMC = MCMC p-value. McAge = population mean-centred age; Blood Age = storage time as blood sample (in years); DNA Age = storage time as DNA sample (in years); Technician (N = 2; contrast = A); BirdID = unique individual identifier; Year = Year of capture; Plate = qPCR plate ID; Row = Row ID on qPCR plate; Units = residuals.

	Post. mode	*95% CrI*	*Effective sample size*	*pMCMC*
Fixed effects				
(Intercept)	**1.605**	**1.109 – 2.012**	**45000**	**<0.0001**
McAge	-0.001	-0.033 – 0.035	**45000**	0.980
Blood Age	**-0.087**	**-0.135 – -0.034**	**44274**	**0.002**
Blood Age^2	**0.003**	**0.000 – 0.005**	**45000**	**0.031**
DNA Age	0.011	-0.049 – 0.064	44159	0.763
DNA Age^2	**-0.005**	**-0.009 – -0.001**	**44161**	**0.023**
Technician(B)	0.070	-0.079 – 0.219	46051	0.357
Random effects				
Animal				
Intercept	0.061	0.043 – 0.093	41653	
Slope	0.078	0.048 – 0.114	40161	
Intercept:Slope	0.028	0.008 – 0.056	39495	
Year	0.094	0.046 – 0.221	45000	
Plate	0.063	0.044 – 0.093	45861	
Row	0.100	0.038 – 0.376	42963	
Units	0.166	0.155 – 0.180	45000	

SI References

1. R. M.Cawthon, Telomere measurement by quantitative PCR. *Nucleic Acids Res.* **30**, 1–6 (2002).
2. R. M.Cawthon, Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res.* **37**, e21–e21 (2009).