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

Heung Ying Janet Chik[1,2], Aaron Sibma[3], Maria-Elena Mannarelli[3,4], Natalie dos Remedios[3,5], Mirre J. P. Simons[3], Terry Burke[3], Hannah L. Dugdale[1§], Julia Schroeder[6§]

[1]Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen 9747AG, the Netherlands.

[2]School of Natural Sciences, Macquarie University, NSW 2109, Australia.

[3]Ecology and Evolutionary Biology, School of Biosciences, University of Sheffield, Sheffield S10 2TN, United Kingdom.

[4]School of Biological Sciences, University of East Anglia, Norfolk NR4 7TJ, United Kingdom.

[5]School of Social Sciences, University of Auckland, Auckland 1010, New Zealand.

[6]Department of Life Sciences, Imperial College London Silwood Park, Ascot SL5 7PY, United Kingdom.

§Shared last authorship

Corresponding authors: Heung Ying Janet Chik (chikhyjanet@gmail.com); Hannah L. Dugdale (h.l.dugdale@rug.nl)

Abstract

Telomeres are a popular biomarker of senescence, as telomere dynamics are linked with survival and lifespan. However, the evolutionary potential of telomere dynamics has received mixed support in natural populations. To better understand how telomere dynamics evolve, it is necessary to quantify genetic variation in telomere length, and how such variation changes with age. Here, we analyzed 2,083 longitudinal samples from 1,225 individuals across 16 years, collected 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 changes with age, reflecting senescence in this population. We found telomere length to be repeatable (14.0%, 95% CrI: 9.1–19.9%) and heritable (12.3%, 95% CrI: 7.5–18.2%); and shows a genotype-by-age interaction, meaning that genotypes differ in their rate of change of telomere length, and 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 highlight the importance of telomere dynamics as a key biomarker of body physiology for the evolution of senescence.

Keywords: Telomere dynamics; Heritability; Genotype-by-age interaction; Quantitative genetics; Senescence

Lay summary

Telomeres are protective stretches of DNA at the ends of chromosomes that shorten over time. Telomere length and telomere shortening have been previously linked with survival, ageing, and fitness, implying it has a potential to be under natural selection, and evolve. Therefore, estimating how much variation in telomere dynamics is contributed by genes, and how this variation changes with age, allows us to better understand the evolution of ageing. Using long-term data from a wild population of house sparrows, we found that telomere length is heritable, and thus has the potential to evolve. We also found that the rate of telomere shortening is also influenced by genes, and that there was an increase in genetic variation of telomere length at older ages. This finding provided direct empirical evidence concerning telomere length, that supports evolutionary hypotheses of ageing. Our study provides insights into the natural selection patterns that give rise to ageing, supports evolutionary predictions for a fitness-related trait, and encourages further investigation into telomere dynamics as a biomarker of ageing.

Introduction

How variation in senescence, the decline in body state with age resulting in death (1), arises, is a central question in evolutionary biology. To quantify senescence and study its evolution, telomere dynamics has become a popular biomarker. Telomeres are highly-conserved, repeating DNA sequences primarily capping the ends of chromosomes (2). Telomeres are important for maintaining DNA integrity, and protect coding DNA from erosion caused by the lagging strand of linear DNA not being fully replicated, i.e. the end-replication problem (3). Thus, in each cell replication cycle, telomeres shorten (4). Telomere shortening can also be accelerated, e.g., by stress exposure (5, 6), but elongation can also occur through telomerase action and other mechanisms (7, 8). However, telomerase is typically suppressed in adult mammalian or human cells (9). When telomeres shorten to a critical length, cell division ceases, and the cell enters a state of senescence (9). The accumulation of senescent cells can result in a decline in tissue function (10). As such, telomere length could reflect the intrinsic state of an individual, and has become a biomarker for senescence. Indeed, while the specific causal mechanism is still unclear (11), increasing evidence has linked short telomeres, and/or telomere shortening, to decreased survival and lifespan in natural populations (12–15), age-related disease and mortality in humans (9, 16), and decreased reproductive output (17). 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}) – heritability – range from 0 to 1 among vertebrate studies (18, 19). 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 (18). Also, the majority of heritability estimates come from human studies, and laboratory animals of controlled ages and environments, limiting our ability to deduce the roles of selection and evolution under natural conditions (20). 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 (21).

Such age-related changes in V_{a} can indicate selection patterns and evolutionary processes that give rise to senescence itself (20). Two non-mutually-exclusive evolutionary hypotheses explain the origin of senescence (22). First, 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 (23). Second, 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 later life would be selected for (24). Both hypotheses are not mutually exclusive and both predict G×A, where selection weakens with increasing 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 (20). However, it is often difficult, and also rarely a main goal, to distinguish between the two theories using a quantitative genetic approach (20). 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 (25–27) but not others (28, 29). Thus far, only a few studies have tested for G×A in telomere length: (30) found significant but low heritability in TL shortening in free-living jackdaws (*Corvus monedula*); (31) found higher heritability in TL change in wild house sparrows (*Passer domesticus*); while (32, 33) did not find support for GxA in captive diary cattle (*Bos taurus*) and wild common terns (*Sterna hirundo*) respectively. Here, we found G×A in telomere length in a wild, isolated house sparrow population, and demonstrated that telomere length senesces as predicted by evolutionary theory.

Results

We used longitudinal data (2,083 samples from 1,225 sparrows, 2000–2015), where the exact age of repeatedly sampled individuals is known (34). 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 was influenced by storage effects, specifically the duration of time that the sample was stored as: a) blood before DNA extraction, and b) DNA before TL measurement (Table S1). TL showed moderate repeatability and heritability (Model 7, individual repeatability = 14.0% (95% CrI: 9.1–19.9%), heritability = 12.3% (95% CrI: 7.5–18.2%), Table S2, Fig. 1). Little variation in TL was explained by the identities of the rearing parents, and by cohort, but capture year accounted for 12.6% (95% CrI 5.7–29.7%; Model 7, Table S2, Fig. 1) of the phenotypic variance. The rates of how fast telomeres shortened with age differed among individuals, evidenced by the statistically significant variance in their intercepts and slopes in our random regression model (Table 1). Individuals that initially had a longer TL showed 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). This means there is a genetic link between having longer telomeres and slower telomere shortening, detected by the 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 3 years of age, and then increased at later ages (Fig. 2, Table S3). This pattern was confirmed by a 'character-state animal model', where genetic variances in TL classified into life stages were high at 'juvenile' stage, lowest at 'young', and increased through 'middle age' and 'old' ages (Table S9).

Discussion

In this study, we investigated genetic and environmental sources of variation in telomere dynamics in a free-living passerine, and provided evidence for individuals differing in the rate of telomere shortening, and that this shortening has a genetic component indicative of G×A. These results support both the mutation accumulation and antagonistic pleiotropy hypotheses that explain the evolution of senescence.

We also showed that TL undergoes senescence in adult wild birds, in line with reports in other natural systems (35–37). Beyond the rapid growth period during early life when telomeres shorten rapidly, 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 (38). However, in our system, telomere length 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 was further supported by our finding that TL was positively correlated with survival independent of age (39).

Our heritability of 12.3% is similar to that found in another house sparrow population (31), but is low compared to the global average among vertebrates (44.9%, (18)), and to some bird species: 99% in zebra finches (*Taeniopygia guttata*, (40)), 81% in tree swallows (*Tachycineta bicolor*, (41)), 77% in jackdaws (*Corvus monedula*, (30)), 65% in common terns (*Sterna hirundo*, (33)), and 48% in great reed warblers (*Acrocephalus arundinaceus*, (42)). However, it is higher than two other wild bird populations: 3.1–8.0% in Seychelles warblers (*Acrocephalus sechellensis*, (43)), and 3.8% in white-throated dippers (*Cinclus cinclus*, (44)). Such inconsistency among studies could have a biological explanation, for example being under stronger selection pressure, which

reduces genetic variation. However, telomere length heritability estimates are also influenced by the laboratory assay used to estimate TL, the statistical methods used to estimate heritability, and potentially the age at sampling (18), all of which differed between these studies. With increasing age 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 (38), and reduce the activity of telomerase, a major telomere lengthening mechanism (45). As such, in contrast to this study on adult telomere length, early-life telomere length (30, 41, 42) could exhibit higher heritability, as the relative contribution from genetic differences would be higher than the environmental differences at this stage (18, but see 43). Parallel to this, TL measurement by qPCR, compared to other methods such as terminal restriction fragment (TRF) methods, introduces higher measurement error, which could also be a cause of higher heritability estimates in studies using the latter method e.g. (30, 33).

Annual stochasticity, e.g. environmental factors that could induce stress, explained a relatively considerable proportion of variance, of 11%. In contrast, the identity of the rearing parents did not explain variation in TL, despite better parental care or foster parental quality being associated with longer offspring telomeres in other species (47, 48). Cohort did not explain much variation in adult telomere length. In our study population, early-environmental effects on juvenile TL, if present, may therefore not carry over past the developmental stage and into adulthood. Storage time also had a significant effect on TL, in line with previous experimental findings in this population (49). Blood and DNA storage conditions influence telomere length measurement - for example, TL varies with blood storage time (50), storage methods (51), DNA concentration (52), and DNA buffer choice (53). In our dataset storage effects are unlikely to influence results, because when we redid the analysis with a dataset that we restricted to blood storage time <5 years we arrived at the same qualitative conclusions (54). Nevertheless, we encourage that researchers be mindful of the storage protocols used in telomere studies, and account for it statistically where possible, and we stress the importance of systematic investigation into the effect of storage conditions on TL.

We provided evidence 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 (46, 55). Much remains unknown about telomere maintenance and repair mechanisms, such as the expression of telomerase. While it is evident that this varies vastly across taxa (56), we do not know much about how increased antioxidant capacity reduces telomere loss (38). Our results emphasize the importance of examining the genetic and environmental influences on these mechanisms, and, on an evolutionary level, the importance of understanding whether the rate of telomere shortening is genetically associated with fitness, as this would mean that there could be selection acting on telomere dynamics.

Testing for G×A allowed us to study changes in genetic variation across ages. V_a in TL increased from the age of 3 years, in agreement with both the mutation accumulation and antagonistic pleiotropy theories of senescence (23, 24). Both theories are not mutally exclusive and assume that selection pressures weaken at older ages, allowing suboptimal genotypes and thus greater genetic variation to remain in the population. While the increase of V_a at older ages could also be a statistical artefact of fitting second order Legendre polynormials, our additional analyses (supplement) provided similar results, supporting this genetic pattern. We also discovered negative genetic covariance in TL between early and late age classes, evidence for antagonistic pleiotropy (20, 57). However, as these negative correlations were not observed throughout all early-late age-class pairs, and were also not present in the character state model, this interpretation should be made with caution.

We detected a decrease in V_a between ages 0 and 2-3 years, contrary to an expected uniform increase in genetic variation in fitness-related traits undergoing senescence (21). 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 mid-life, causing higher genetic variance in both early and late life (21). However, we consider this explanation unlikely, as telomere lengthening currently lacks support in birds (but see (31, 58), and e.g. (59, 60) in mammals). Furthermore, lengthening could easily be masked by methodological effects such as measurement error (60), leukocyte composition changes, and storage time effects, which significantly influenced TL in our dataset. It is more likely that the decrease in V_a during early life pertained to mortality risks. In the Lundy house sparrows, mortality is higher in both early (0-1 years) and later ages (5-7 years) (39, 61). In addition, independent of age, TL is positively linked with survival (39). These two findings together suggest a genetic bottleneck leading up to age 2, where young birds with longer TL survive to breeding ages, leading to lower genetic variation in TL around that age.

To better understand how selection shapes genetic variation in telomere length, an important step would be to examine the links between telomere dynamics and fitness. There is evidence that telomere length is indeed positively associated with reproductive success in the Lundy house sparrows (39), supporting that the GxA patterns observed here could be a result of selection, and thus stressing the importance of telomere dynamics in the studying the evolution of senescence.

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 (62). We collected telomere, life-history and pedigree data from a free-living, 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 (34), 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 birds breeding in nestboxes, including the identities of the parent of each brood, the offspring identities and hatch dates. This allowed us to determine the exact age of each bird at sampling. A small minority of birds fledged from inaccessible nests, and we captured them 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. Due to the mobile nature of birds it is typically difficult if not impossible to gather such precise age and death estimate in natural populations. Therefore, the above listed chracteristics of our study populations render our telomre dynamic estimates unusually precise.

To quantify telomere length and assign genetic parentage, we collected blood samples repeatedly from individual birds, systematically at two and 12 days of age, during their first winter, and on every subsequent capture. Previous analyses of these data suggested that all birds were equally likely to be caught and sampled (63). We stored blood samples in 96% 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 (64). All animal procedures were approved by the 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 (65). 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 (66). For details, see the supporting text in the 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 was not correlated with the final T/S ratios (49). Plates were run by two technicians (MEM ran 52 plates and NdR ran 30 plates). Mean qPCR amplification efficiencies for the telomeric product and the single reference gene were 90.7% (range: 71 – 109%) and 94.5% (range 76 – 119%) respectively (49). From a separate experiment on the effects of storage time on TL, we selected a subset of 80 samples, and either re-extracted DNA from blood, or re-assayed telomeres from existing DNA. From these, we calculated inter-plate repeatability to be 0.49 (s.e. = 0.07) (49). Our final full dataset consisted of 2,083 telomere length measurements from 1,225 birds, 476 of which have at least two telomere length observations. Further telomere dataset summaries are provided in Tables S4 and S5.

Genetic pedigree construction

We used up to 23 house sparrow microsatellite markers (67) to construct a genetic pedigree for individuals born 1995–2019, using Cervus 3.0 (68). 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 (69) (69)). 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 1,321 birds, with 1,196 assigned maternities, 1,197 assigned paternities, and a maximum pedigree depth of 16 generations (Fig. S2). Pedigree statistics were calculated using *pedantics* 1.7 (70) and are summarized in Table S6.

Statistical analysis

All analyses were carried out in R 4.0.3 (71). Regression models were built using the Bayesian package *MCMCglmm* 2.29 (72). 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 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 the 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 (73). 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 TL decreases with sample storage time in our dataset (49), 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: $W = 233714$, $p<0.001$), 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, plate ID and row ID to account for technical variance among qPCR plates and among row positions on each plate (74). 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 data and sex had no effect (see Results). 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 among-individual slopes could lead to a biased estimation of the individual variances in the random effects structure (75), 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 (73). The two slopes were statistically significantly different from each other (posterior mode for BtAge = 0.063, 95% CrI = 0.027–0.103).

(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 variances explained by random effects are conditioned on fixed effects, and hence not including fixed effect variation in calculating V_{t} could lead to underestimation of V_{t} and overestimation of repeatability and heritability (76). 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 (77), 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 explained negligible variances (see results). The final model equation for the RRM was 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 (27) and (78), 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 (78). 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. This RRAM successfully converged, but estimated similar genetic and permanent environmental variances (Table 2). Therefore, 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 first 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 $\Phi_{ij} = \varphi_0(age*_i)$ (78).

To verify the RRAM, we further fitted a 'character-state model', where age-specific TL measurements were treated as correlated sub-traits. We first corrected TL measurements by fitting a mixed model with BloodAge, DNAAge, and their square terms, and technician ID as fixed predictors, and plate ID as a random predictor. The residual TL values were then pooled into four stages: 1) "juvenile" (age 0); 2) "young" (ages 1 and 2); 3) "middle age" (ages 3 and 4); and 4) "old" (ages 5 or above) for each individual. When an individual was sampled more than once within each stage, we took the mean of these TL measurements. We then fitted a multivariate animal model in MCMCglmm, where measurements from the four stages were fitted as multivariate response variables, and the animal and BirdID terms as random variables, allowing genetic variance and covariances among stages to be estimated.

Author Contributions: JS, HLD and TB conceived the study, and all authors contributed to the development of the study. AS, MEM, NDR and MJPS collected the telomere data, and JS and TB curated the long-term life-history data. HYJC generated the study dataset, performed the statistical analyses and drafted the manuscript, with input from JS and HLD. All authors provided comments on the manuscript and agreed on the final version of the manuscript to be submitted for publication.

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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

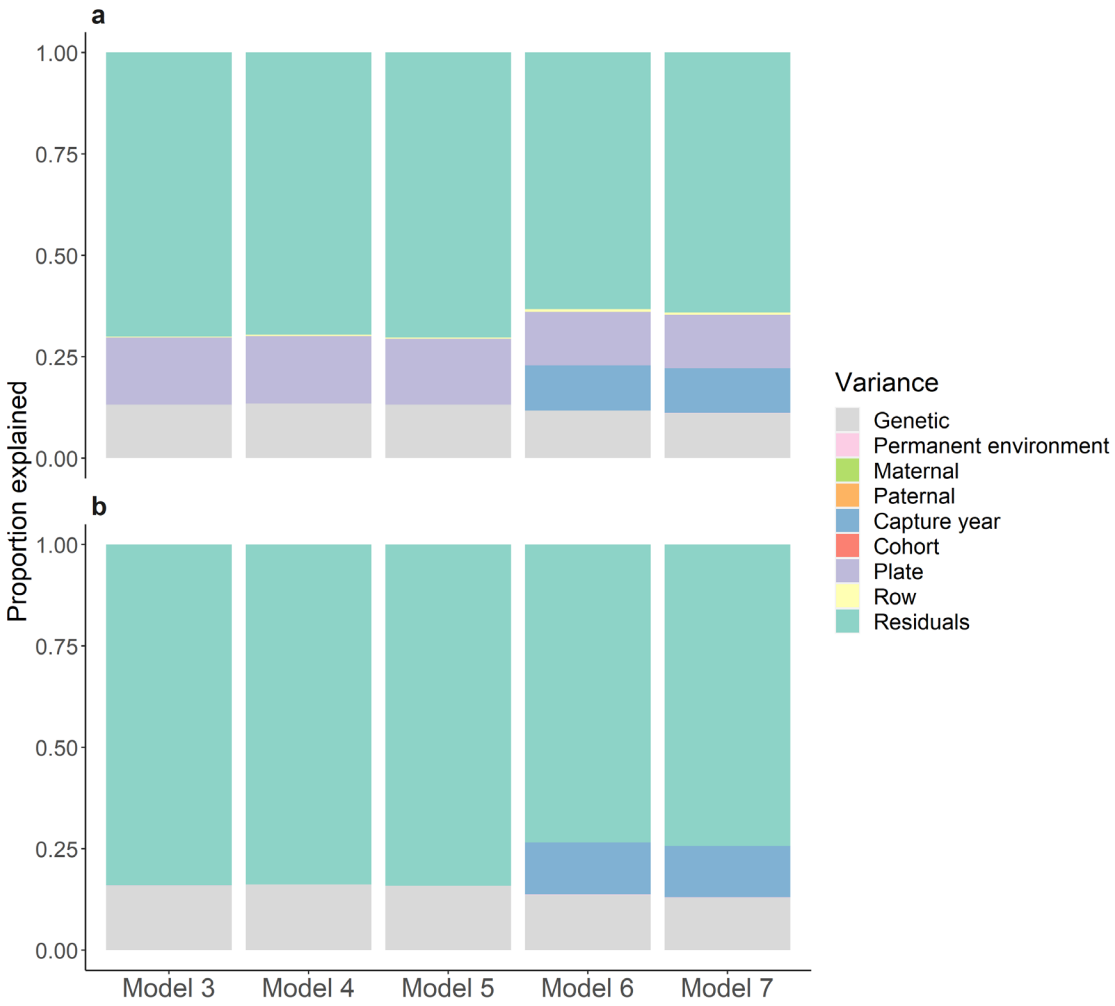

Figure 1. Variance components from a series of 'animal' models (Models 3–7) to estimate sources of variation in telomere length in the Lundy house sparrow population sampled in 2000–2015: (a) the proportions of all fitted random variables, and (b) proportions of biologically relevant random variables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

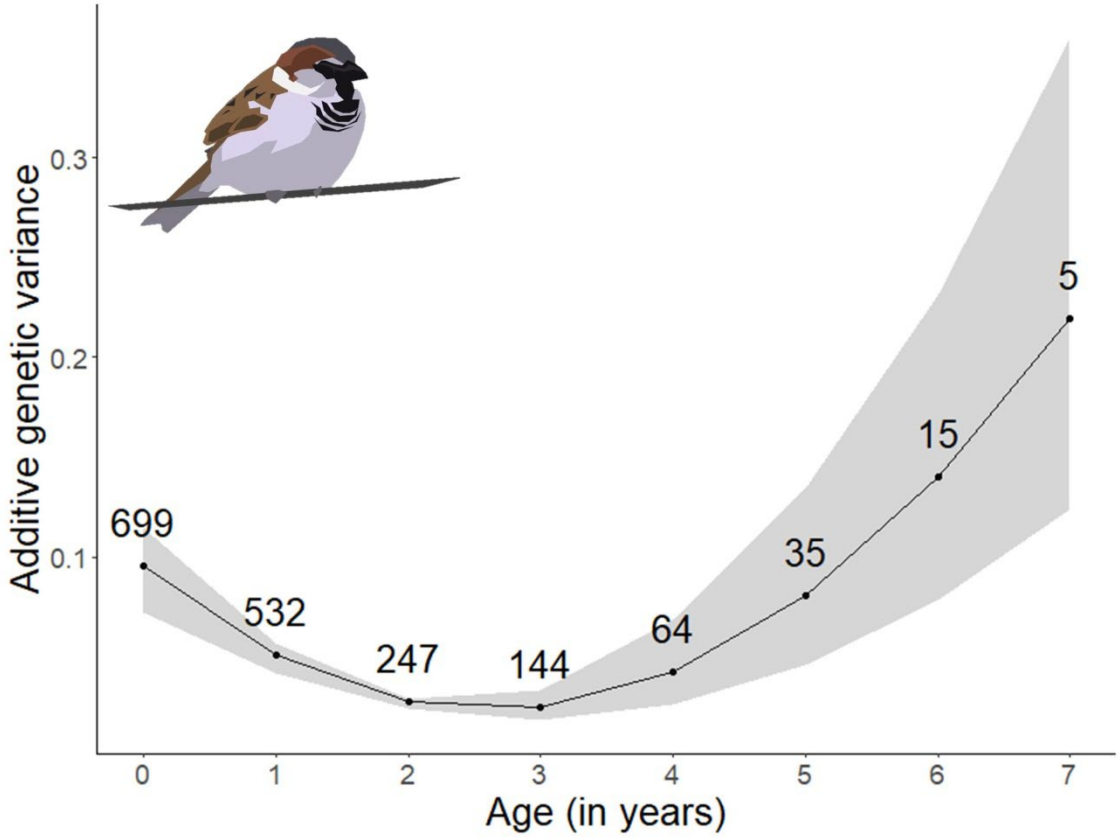

Figure 2. The quadratic relationship between additive genetic variance of telomere length and age in Lundy house sparrows sampled in 2000–2015. Black dots represent point estimates of additive genetic variance for each age class (0–7), and the shaded area around each dot represents the 95% CrI of the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

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)	**1.584**	**1.340 – 1.807**	**180000**	**<0.0001**
McAge	-0.008	-0.033 – 0.017	179123	0.861
Blood Age	**-0.103**	**-0.144 – -0.061**	**180000**	**<0.0001**
Blood Age^2	**0.003**	**0.001 – 0.006**	**180000**	**0.008**
DNA Age	0.019	-0.030 – 0.070	180000	0.434
DNA Age^2	**-0.007**	**-0.011 – -0.003**	**180000**	**0.001**
Technician (B)	0.018	-0.114 – 0.147	180000	0.792
Random effects				
BirdID				
Intercept	0.061	0.042 – 0.090	31670	
Slope	0.081	0.052 – 0.124	28248	
Intercept: Slope	**0.030**	**0.011 – 0.061**	**26118**	
Year	0.030	0.013 – 0.087	180000	
Plate	0.036	0.024 – 0.055	180000	
Row	0.001	0.000 – 0.008	180000	
Units	0.161	0.148 – 0.175	143582	

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)	**1.642**	**1.204 – 2.032**	**43883**	**<0.0001**
McAge	-0.007	-0.056 – 0.044	45000	0.851
BloodAge	**-0.095**	**-0.150 – -0.045**	**45000**	**0.001**
BloodAge^2	**0.003**	**0.000 – 0.006**	**44819**	**0.018**
DNAAge	0.031	-0.034 – 0.084	45658	0.403
DNAAge^2	**-0.007**	**-0.012 – -0.003**	**45000**	**0.002**
Technician (B)	0.016	-0.148 – 0.162	45000	0.954
Random effects				
Animal				
Intercept	0.062	0.042 – 0.094	39970	
Slope	0.084	0.052 – 0.126	39836	
Intercept:Slope	**0.034**	**0.015 – 0.071**	**38176**	
BirdID				
Intercept	0.057	0.039 – 0.085	41085	
Slope	0.078	0.051 – 0.122	40070	
Intercept:Slope	**0.036**	**0.017 – 0.069**	**39796**	
Year	0.092	0.046 – 0.237	45000	
Plate	0.053	0.037 – 0.076	45841	
Row	0.093	0.040 – 0.343	47518	
Units	0.150	0.137 – 0.162	45000	