

High heritability of telomere length and low heritability of telomere shortening in wild birds

Christina Bauch¹  | Jelle J. Boonekamp¹  | Peter Korsten²  | Ellis Mulder¹  | Simon Verhulst¹ 

¹Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands

²Department of Animal Behaviour, Bielefeld University, Bielefeld, Germany

Correspondence

Christina Bauch, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands.
Email: christinabauch@gmx.de

Present address

Jelle J. Boonekamp, Institute of Biodiversity Animal Health & Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

Funding information

Nederlandse Organisatie voor Wetenschappelijk Onderzoek, Grant/Award Number: 823.01.009; Deutsche Forschungsgemeinschaft, Grant/Award Number: BA 5422/1-1

Abstract

Telomere length and telomere shortening predict survival in many organisms. This raises the question of the contribution of genetic and environmental effects to variation in these traits, which is still poorly known, particularly for telomere shortening. We used experimental (cross-fostering) and statistical (quantitative genetic “animal models”) means to disentangle and estimate genetic and environmental contributions to telomere length variation in pedigreed free-living jackdaws (*Corvus monedula*). Telomere length was measured twice in nestlings, at ages 4 ($n = 715$) and 29 days ($n = 474$), using telomere restriction fragment (TRF) analysis, adapted to exclude interstitial telomeric sequences. Telomere length shortened significantly over the nestling period (10.4 ± 0.3 bp day⁻¹) and was highly phenotypically ($r_p = 0.95 \pm 0.01$) and genetically ($r_G > 0.99 \pm 0.01$) correlated within individuals. Additive genetic effects explained a major part of telomere length variation among individuals, with its heritability estimated at $h^2 = 0.74$ on average. We note that TRF-based studies reported higher heritabilities than qPCR-based studies, and we discuss possible explanations. Parent–offspring regressions yielded similar heritability estimates for mothers and fathers when accounting for changes in paternal telomere length over life. Year effects explained a small but significant part of telomere length variation. Heritable variation for telomere shortening was low ($h^2 = 0.09 \pm 0.11$). The difference in heritability between telomere length (high) and telomere shortening (low) agrees with evolutionary theory, in that telomere shortening has stronger fitness consequences in this population. Despite the high heritability of telomere length, its evolvability, which scales the additive genetic variance by mean telomere length, was on average 0.48%. Hence, evolutionary change of telomere length due to selection is likely to be slow.

KEYWORDS

early-life, inheritance, life-history, quantitative genetics, senescence, ageing

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Molecular Ecology* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

Individuals differ in lifespan and other fitness components, and identification of the molecular and physiological traits associated with fitness is a useful step in deciphering ecological and evolutionary dynamics of life histories. Telomere length and telomere shortening are such traits, because a shorter telomere length generally predicts a lower survival probability (meta-analyses in humans: Boonekamp et al., 2013; and wild vertebrates: Wilbourn et al., 2018) as does higher telomere shortening (in our study species: Salomons et al., 2009, Boonekamp et al., 2014; and other species: Barrett et al., 2013, Sheldon et al., 2021). Telomere length and telomere shortening are therefore increasingly being used as biomarkers of ageing and phenotypic quality across research areas such as epidemiology, ecology and evolutionary biology (Monaghan et al., 2018). However, to what extent both genetic and nongenetic factors contribute to among-individual variation in telomere length, and in particular to telomere shortening, remains poorly understood.

Telomeres consist of evolutionarily conserved, noncoding DNA sequence repeats (Blackburn, 1991) that together with the shelterin protein complex form the ends of chromosomes (de Lange, 2005) and contribute to genome stability (O'Sullivan & Karlseder, 2010). Telomeres are dynamic structures, in that their length shortens with age due to incomplete replication during cell division, which can be accelerated by DNA- and protein-damaging factors and attenuated or counteracted by maintenance processes (Chan & Blackburn, 2004). When telomeres reach a critically short length, the cell undergoes replicative senescence or apoptosis (Chan & Blackburn, 2004). In this way, telomere length and telomere shortening may play a causal role in senescence. Regardless of the question of causality, telomere length and telomere shortening are of interest as proxies for general health and senescence processes (Young, 2018).

To understand how telomere length and telomere shortening may play a role in shaping life histories from an evolutionary perspective, it is necessary to quantify their additive genetic variance on which natural selection can act. However, heritability estimates of telomere length differ markedly between studies, and this variation is not yet understood. To what extent variation in telomere length is due to inheritance or driven by the environment is therefore still under debate (Atema et al., 2015; Benetos et al., 2019; Broer et al., 2013; Dugdale & Richardson, 2018). Differences in the genetic background of populations, temporal-spatial variation of environmental influences, and methodological differences including limitations to separate genetic and environmental effects can all underlie variation in heritability estimates between studies (Becker et al., 2015; Dugdale & Richardson, 2018).

Telomere length at any given age is defined by the initial telomere length transferred through the parental gametes and the change in telomere length from the zygote stage onwards, which varies under the influence of both genes and the environment. For example, telomere shortening is generally accelerated with increased exposure to various (environmental) stressors; Angelier et al., 2018; Boonekamp

et al., 2014; Kotrschal et al., 2007; McLennan et al., 2016; Seeker et al., 2021). Telomere shortening is typically faster early in life (Benetos et al., 2019; Fairlie et al., 2016; Salomons et al., 2009; Spurgin et al., 2018), potentially resulting from a greater susceptibility of telomeres to environmental and genetic effects in early life. Thus, exposure to environmental influences, age-dependent sensitivity to environmental effects and age-dependent gene expression all contribute to telomere length variability.

Most studies of telomere length heritability have measured telomere length at different ages in different individuals (reviewed in ref. Dugdale & Richardson, 2018), which affects the comparability of telomere length between individuals due to variation in exposure to environmental effects. In this way, sampling at variable ages will bias telomere length heritability estimates to an unknown extent, even when taking age variation into account in the estimation process. On the other hand, a shared environment among related individuals (e.g., due to parental care or location) can increase phenotypic similarity (e.g., in telomere length) and thereby inflate heritability estimates (Kruuk & Hadfield, 2007). Moreover, similarity in telomere length between parents and offspring can potentially arise in multiple ways, via additive genetic effects on gamete telomere length and telomere length regulation (Codd et al., 2013; Soerensen et al., 2012), epigenetic effects (e.g., mediated by paternal age; Bauch et al., 2019; Eisenberg, 2019; Noguera et al., 2018), and via early-life parental effects of genetic and nongenetic origin (Crisuolo et al., 2017; Marasco et al., 2019). Quantifying telomere length heritability is therefore challenging.

Quantitative genetic “animal models” are a powerful statistical tool to disentangle genetic from environmental effects, which makes use of all relatedness information available in a pedigree (Wilson et al., 2010). As this is a “data-hungry” method, the number of studies using it to investigate causes of telomere length variation is currently limited, and results are highly variable (reviewed in refs. Dugdale & Richardson, 2018; Froy et al., 2021; Sparks et al., 2021). Moreover, all except one study on wild vertebrates (Vedder et al., 2021) measured telomeres using qPCR (quantitative polymerase chain reaction), which pools all telomeric sequences in the genome (located both terminally and interstitially; Nussey et al., 2014). It is not clear therefore how the currently available estimates relate to the heritability of the length of the terminal telomeres, which are subject to age- and stress-related shortening. Even less is known of genetic variation in telomere shortening, with one study on human twins reporting a heritability of 0.28 (95% confidence interval [CI] 0.16–0.44; Hjelmborg et al., 2015).

Heritability (h^2) is estimated as the ratio between the additive genetic variance (V_A) and the total phenotypic variance (V_P) $h^2 = V_A/V_P$ (Falconer & Mackay, 1996), and as such, low heritability can result from low additive genetic variance, but also from high total variance (e.g., because of large environmental effects). The evolutionary potential of a trait is dependent on its additive genetic variance, which scaled to the mean of the trait yields a metric known as the evolvability (Houle, 1992). Given the evidence for survival selection on both telomere length and telomere

shortening (Barrett et al., 2013; Boonekamp et al., 2013, 2014; Sheldon et al., 2021; Wilbourn et al., 2018), their evolvability is of interest. However, we are aware of only one study estimating the evolvability of telomere length that was based on the absolute size of terminally located telomere length measured in base pairs. This study on captive field crickets found evolvability to be low despite high heritability (Boonekamp et al., 2021).

The aim of the present study was to quantify the additive genetic and environmental contributions to the variability of telomere length among and within individuals to estimate the heritabilities of telomere length and telomere shortening and the potential for telomere length to evolve over time in a population of free-living wild jackdaws (*Corvus monedula*). To this end, we measured telomere length in erythrocytes at the age of 4 days and for the majority of individuals again at 29 days (contingent primarily on their survival), using the golden-standard technique telomere restriction fragment (TRF) analysis adapted to measure terminally located telomeric sequences only (Nussey et al., 2014; Salomons et al., 2009). To disentangle genetic and environmental causes of variation in telomere length and shortening we (i) applied experimental cross-fostering and (ii) made use of the available multigenerational pedigree information by running uni- and bivariate “animal model” analyses (Wilson et al., 2010). Using these methods, we estimated the heritabilities of telomere length and telomere shortening, and—to test for potential age-dependent genetic effects—the genetic correlation of telomere lengths measured at different ages. We additionally performed parent–offspring regressions to assess the relative maternal and paternal contributions to the variation in offspring telomere length, while also taking into account effects of paternal age at conception (Bauch et al., 2019), and compared early-life telomere shortening between parents and offspring. Lastly, we estimated the additive genetic variance of telomere length in relation to absolute trait size (i.e., evolvability) to assess the potential for telomere length to evolve in our population. As telomere length has been shown to differ between the sexes in several species (Barrett & Richardson, 2011; Bauch et al., 2020), we also tested for sex differences in telomere length and telomere shortening in this study.

2 | MATERIAL AND METHODS

2.1 | Study population

We studied a jackdaw (*Corvus monedula*) population breeding in nest-box colonies located south of Groningen, The Netherlands (53°14'N, 6°64'E), which has been under investigation since 1996. Birds in our study population are ringed with numbered metal rings and colour rings shortly before fledging or as immigrants at first breeding in the study's nestboxes. Breeders are highly site faithful and all breeding birds are identified by their unique colour ring combinations via telescope, photo or video camera. Jackdaws breed monogamously with low divorce rates and very rare extra-pair paternity (Henderson

et al., 2000; Liebers & Peter, 1998). However, due to partner death, about 50% of the adults in our data set had two or more partners and the population comprises full as well as half-siblings produced over multiple years. From 1 day before the expected hatching date the nestboxes were checked daily for hatchlings. Freshly hatched chicks were marked by specific combinations of clippings of the tips of the toenails for identification until ringing. Exact ages were known for jackdaws native to the study's nestboxes. Immigrants were assigned an age of 2 years when breeding for the first time, which is the modal age at recruitment in our population. The sex of all jackdaw parents and the majority of chicks has been identified either molecularly (Griffiths et al., 1998) or by behavioural observations and cross-reference with breeding partners.

2.2 | Pedigree

The pruned pedigree consists of 1007 relatedness-informative individuals, whereof 715 individuals held data on telomere length at the age of 4 days of which 474 individuals additionally held telomere length data when 29 days old. The pedigree spans six generations for known telomere length of either age. For details on relationships in the pedigrees see Table S1 and Figure S1. Summary statistics and pedigree images were generated using the R-package PEDANTICS (Morrissey & Wilson, 2010).

2.3 | Cross-fostering

To experimentally disentangle additive genetic from early-life parental effects we performed two types of cross-fostering manipulations in our study population. In 2015 and 2016 in a subset of the nestboxes ($n = 58$ of 126 nests) complete clutches were exchanged between nests during midincubation. Nests were matched for clutch size and laying date (± 1 day), but in all other respects cross-fosters were performed randomly. In this way, foster parents already incubate and take care of the nestlings as soon as they hatch. Additionally, in all years, brood sizes were manipulated in most nests, with nestlings transferred between nests when the oldest nestling was 4 days old. Age-matched broods were reduced by removing three nestlings and adding one nestling and enlarged by adding three nestlings and removing one (for details, see Boonekamp et al., 2020). Consequently, manipulated broods (i.e., between first and second telomere length measurement) comprised genetic and nongenetic offspring of parents and, thus, both genetic and nongenetic siblings. The multigenerational pedigree included individuals with various degrees of relatedness, which experienced the same or different rearing environments. The cross-fostering experiments add additional information to the data set by adding full siblings reared in different broods. For an overview of the study design and sample distribution over the different treatments see Figure 1.

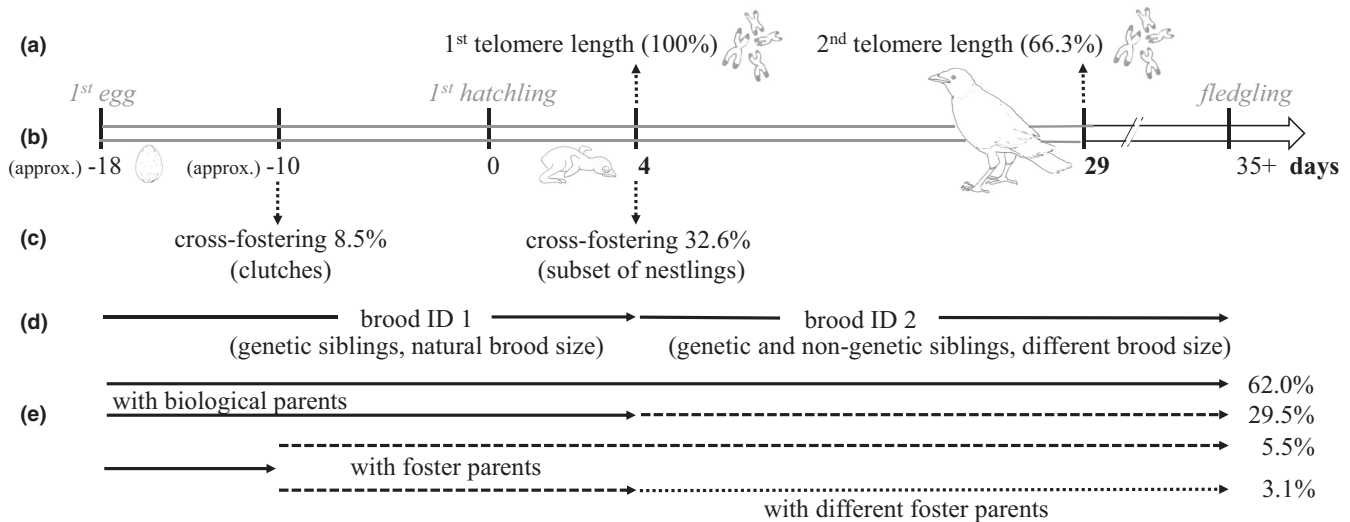


FIGURE 1 Study design. (a) Blood sampling for telomere length measurement: 1st sample $n = 715$, 2nd sample $n = 474$ (subset of individuals). (b) Timeline of a jackdaw brood from egg to fledging. (c) Cross-fostering experiment, with exchange of entire clutches and exchange of a subset of nestlings between broods. (d) Brood ID for the individual: the composition of the brood changes when the oldest nestling of a brood is 4 days old. (e) Depending on the cross-fostering performed, the nestlings remain for more or less time with the biological parents, and have one or two different foster parents during the nestling phase (respective parent ID in the models). (Percentages, rounded to first decimal, are for the 715 nestlings sampled at least once. The percentages in (e) change by a maximum of 1.9% if only the subset of 474 repeatedly sampled nestlings is considered.)

2.4 | Blood sampling and measuring of telomere length

Telomere length was measured in blood samples taken between 2005 and 2016 from 715 individuals at the maximum age of 4 days (mean \pm SD = 3.7 ± 0.6 days) and again 25 days later in a subset of 474 individuals (age 29 days; Figure 1). This interval between repeated telomere measurements of nestlings covers ~70% of their time in the nest (Röell, 1978). Fathers were caught and blood sampled when the brood was at least 14 days old, contributing telomere data of 82 fathers at conception across multiple years to the data set. Samples were stored in 2% EDTA buffer at $4-7^{\circ}\text{C}$ and within 3 weeks snap frozen in a 40% glycerol buffer for permanent storage at -80°C . Telomere length of nucleated erythrocytes was measured performing TRF analysis under nondenaturing conditions using pulsed-field gel electrophoresis as in Salomons et al. (2009). See Text S1 for details, including a gel image (Figure S2). A sample of an individual consists of a characteristic telomere length distribution from erythrocytes of different ages and different chromosomes within cells. The individual average of this telomere length distribution was used in the present analyses (Salomons et al., 2009). Samples were distributed over 57 gels. Samples from nestlings of the same brood were partly spread over different gels. Repeated samples (two ages) from individuals were run on the same gel. As a quality index for intergel comparability, we calculated the coefficient of variation of one control sample from a 29-day-old jackdaw run on 26 gels, which was 6%, and of one control sample of a goose, with a similar telomere length distribution in a similar range, run on 31 other gels, which was 7%. Thus, intergel measurement accuracy remained similar over time. The high phenotypic correlation of

telomere length within individuals measured at the two ages that we find in our data set ($r_p = 0.95 \pm 0.01$, $n = 474$, Table 1) reflects a high intragel repeatability for telomere length, since repeated samples were always on the same gel. This estimate has the advantage of covering the complete method procedure between blood sampling and final calculation of telomere length for a large data set.

2.5 | Statistical analyses

First, we investigated effects of age and sex on telomere length and telomere shortening. We ran linear mixed-effects models with telomere length as the dependent variable, age as a factor to investigate telomere shortening over the interval of 25 days and bird ID as a random effect. Gel ID was included as a random effect to control for methodologically induced variance. With a log-likelihood ratio test, comparing models with and without bird ID, we tested for significant between-individual variation of telomere length. Further, by including the factor sex and its interaction with age in the model, we tested for potential sex differences in telomere length and telomere shortening.

Second, we ran a series of univariate quantitative genetic “animal models” (Wilson et al., 2010) using early-life telomere length data and pedigree information holding various degrees of kinship to partition the total phenotypic variance in early-life telomere length (V_p) measured at the ages of 4 or 29 days into its additive genetic (V_A), residual (V_R) and varying other components of potential relevance: biological mother ID (V_{BM}) estimates the variance in telomere length among nestlings from different mothers over and above the variance that is attributable to additive genetic effects,

TABLE 1 Results from a bivariate animal model analysis on telomere length (TL) measured at 4 days old in 715 individuals and again at 29 days old in a subset of 474 individuals. (a) Fixed effects, variance components and covariances with regard to telomere length. Brood ID changed due to experimental manipulation at age 4 days and accordingly the respective brood ID (B1, B2) was fitted for the specific telomere length measure (TL4, TL29). The variances for random effects were estimated for telomere lengths at each age and for the covariance between the two telomere lengths, except for biol. mother ID and gel ID, which each have a common variance for telomere length of both ages (see also Table S8). (b) Heritability estimates for telomere length and telomere shortening (Δ TL) derived from the bivariate model. (c) Correlations between TL at ages 4 and 29 days. For study design see Figure 1

(a)					
Fixed effects	TL 4 days Estimate (SE)	TL 29 days Estimate (SE)	F	df	p
Intercept	7,118.0 (75.0)	6,884.0 (79.9)			
Paternal age	-25.1 (13.4)		3.5	1,269.7	.062
		-30.2 (14.2)	4.5	1,286.8	.034
		TL 4 days	TL 29 days		Proportion of variance
Random effects	Variance (SE)	Proportion of variance	Variance (SE)		
Additive genetic	203,924.5 (40,969.8)	0.603	243,951.3 (46,501.0)	0.660	
Biological mother ID	13,850.6 (15,665.8)	0.041	13,850.6 (15,665.8)	0.037	
Brood ID (B1) until age 4 days	901.3 (1,824.7)	0.003			
Brood ID (B2) age 4–29 days			2,303.6 (1,692.4)	0.006	
Year	21,123.0 (15,299.1)	0.062	23,862.1 (17,640.3)	0.065	
Gel ID	50,495.6 (14,266.9)	0.149	50,495.6 (14,266.9)	0.137	
Residual	47,691.6 (22,173.6)	0.141	34,899.0 (25,432.0)	0.094	
Random effects					Covariance (SE)
Additive genetic					222,869.8 (43,118.3)
Year					21,730.0 (16,128.6)
Residual					29,961.0 (23,195.6)
(b)					
Heritability h^2 (SE)					
	TL 4 days	TL 29 days	Δ TL		
variance due to gel effect included	0.603 (0.107)	0.660 (0.108)	see Table S8		
biological variance only (gel effect excluded)	0.709 (0.121)	0.765 (0.121)	0.088 (0.114)		
(c)					
Correlations between TL at ages 4 and 29 days					
Phenotypic r (SE)					Additive genetic r (SE)
0.954 (0.006)					0.999 (0.006)

or in other words it accounts for early-life maternal effects (before eventual cross-fostering). The telomere length in offspring from the same biological mother may be more similar as compared to offspring from other mothers due to mother-specific effects, if, for example, egg content affects offspring telomere length (Hausmann et al., 2012). Similarly, biological father ID (V_{bF}), and mother or father ID after experimental cross-fostering could affect telomere length of nestlings by parental care effects (e.g.,

Vedder et al., 2018). Year (V_Y) was added to account for potential year effects on offspring telomere length. Brood ID (V_b) estimates the variance that can be attributed to the shared environment of brood mates. In the model with telomere length at age 4 days we included brood ID until the age of 4 days (only genetic siblings and natural brood size, B1; Figure 1). In the model with telomere length at age 29 days we additionally included brood ID of the following 25 days (genetic and nongenetic siblings, manipulated brood size,

B2). To account for measurement differences between gels, we always included gel ID as a random effect (V_{gel}). As a fixed effect we included biological father age (Bauch et al., 2019). The fixed effect of sex was not significant and therefore excluded from the models. As we measured telomere length for the entire brood when the oldest nestling was 4 days old, while there is age variation within broods due to asynchronous hatching, we tested whether the exact age of the nestling had an effect on telomere length. However, this was not the case and therefore this predictor was also excluded from the models. Statistical significance of fixed effects was determined using Wald tests and of random effects using log-likelihood ratio tests, comparing models with and without the specific random effect.

Third, we ran bivariate models with telomere length of both nestling ages. We included the same random effects as in the univariate model analysis. The additive genetic and year random effects were fitted with an unstructured variance-covariance matrix (i.e., for each random effect the variance was estimated for telomere length at each age as well as the covariance between the two telomere lengths). We initially included the random effect gel ID in the same manner, but this model structure prevented us from reliably estimating the variance for brood ID B1 (until age 4 days), possibly due to over-parameterization issues (Table S8). We therefore included gel ID as a common random effect estimating the common variance for the two ages in the final model. A comparison of the results from the two models (Table 1; Table S8) showed that the way the variance was fitted for gel ID had virtually no effect on the estimated genetic and phenotypic correlations, or the estimated heritability for telomere shortening. We also included biological mother ID estimating the common variance, because it did not significantly affect the variation in telomere length at any of the two ages. The statistical significance of fixed effects (paternal age) was determined using Wald tests.

Fourth, we ran a univariate model with telomere shortening as the dependent variable, including again the same random effects. With log-likelihood ratio tests, we determined the statistical significance of random effects on telomere shortening between the ages of 4 and 29 days.

We calculated narrow sense heritability (h^2) estimates for telomere length as:

$$h^2 = \frac{V_A}{(V_P - V_{\text{gel}})}$$

excluding variation due to gel effects (V_{gel}) unless stated otherwise. For a more comprehensive understanding of the h^2 -estimate, which can be affected by fixed effects (Wilson, 2008), we reran the main univariate model with random effects only.

From the bivariate model results (Table 1) we additionally calculated the heritability estimate of telomere shortening, the phenotypic and additive genetic correlation between telomere lengths at the two ages, the coefficients of additive genetic variance of telomere length as well as the evolvabilities as follows.

To estimate the heritability (h^2 , see formula above) for telomere shortening ($TL_{29} - TL_4$), the additive genetic variance (V_A) for telomere shortening was derived from the variance-covariance matrix:

$$V_{A29-4} = V_{A29} + V_{A4} - 2 * \text{cov}_A$$

where V_A is the additive genetic variance at age 4 or 29 days and cov_A is the additive genetic covariance between telomere length measured at age 4 and 29 days.

The genetic correlation r_G between telomere length at ages 4 and 29 days was calculated as:

$$r_G = \frac{\text{cov}_A}{\sqrt{V_{A4} * V_{A29}}}$$

The phenotypic correlation r_P was calculated as:

$$r_P = \frac{(\text{cov}_A + \text{cov}_R)}{\sqrt{(V_{A4} + V_{R4})(V_{A29} + V_{R29})}}$$

with cov_R as the residual covariance between telomere length measured at age 4 and 29 days and V_R as the residual variance at age 4 or 29 days.

The coefficient of additive genetic variance of telomere length at both time points was calculated as:

$$CV_A(x) = 100 * \frac{\sqrt{V_A}}{\bar{x}}$$

where x is telomere length and \bar{x} its mean (Houle, 1992). Whether additive genetic variances differ at the two ages was tested with a log-likelihood ratio test comparing bivariate models with constrained common variance and unconstrained variances.

The relative evolvability I_A of telomere length at both time points was calculated as:

$$I_A = \frac{V_A}{\bar{x}^2} = \left(\frac{CV_A}{100} \right)^2$$

(Houle, 1992), multiplied by 100 to express it as a percentage.

Lastly, for comparison with the animal model results, we applied parent-offspring regressions using linear mixed-effects models of early-life telomere length (age 4 days) and telomere shortening during the nestling period (between age 4 and 29 days, ΔTL) between parents and their offspring. We ran these analyses for mothers and fathers separately as data on both parents simultaneously were available in too few cases. When regressing offspring telomere length on father telomere length we ran an additional model that included father age as a fixed effect, as telomere length of consecutive offspring declines as fathers age (Bauch et al., 2019). Additionally, we calculated a father-offspring regression with father telomere length measured in the year of offspring conception. As the data set contains measures of telomere length or telomere shortening of

offspring of the same parents in multiple years and telomere length was analysed on different gels, we added parent ID, brood ID, year and gel ID as random effects.

All statistics were run in R (R Development Core Team, 2017) using ASREML-R software (versions 3 and 4; VSN International) and R-packages LME4 (Bates et al., 2015) and LMERTEST (Kuznetsova et al., 2017).

3 | RESULTS

3.1 | Descriptive statistics

Telomere length at the age of 4 days was on average 7,039 bp ($SD = 591$ bp, $n = 715$) and at the age of 29 days on average 6,729 bp ($SD = 588$ bp, $n = 474$; Figure 2). Telomeres shortened significantly within individuals over the measurement interval of 25 days by on average 260 bp ($SD = 171$ bp; $t = -33.18$, $p < .001$, $n = 474$, Table S2A). Telomere length differed significantly between individuals ($\chi^2 = 986.17$, $p < .001$, Table S2A), and was highly phenotypically correlated within individuals between ages 4 and 29 days ($r_p = .954 \pm .006$, $n = 474$, $p < .001$, Table 1, Figure 2). The sexes differed neither in telomere length (Table S2B) nor in telomere shortening (Table S2C).

3.2 | Animal model analyses

The uni- and bivariate animal model analyses revealed that the major part of the variation in telomere length at the age of 4 and 29 days was explained by additive genetic effects (Table 1; Tables

S3–S8; Figure 3). The narrow sense heritability estimates for telomere length measured at the ages of 4 and 29 days, derived from the bivariate model, were $h^2 \pm SE = 0.71 \pm 0.12$ and 0.77 ± 0.12 , respectively (Table 1). Heritability estimates based on respective univariate models, containing the same sample sizes, but not taking the relationship of repeatedly measured telomere length within individuals into account, were $h^2 \pm SE = 0.63 \pm 0.13$ (lower) for telomere length measured at the age of 4 days (Table S3) and $h^2 \pm SE = 0.88 \pm 0.13$ (higher) for telomere length measured at the age of 29 days (Table S6). Including paternal age as a fixed effect in the model did not significantly affect the heritability estimate for telomere length at age 4 days ($h^2 \pm SE = 0.63 \pm 0.13$, with paternal age, Table S3, vs. $h^2 \pm SE = 0.67 \pm 0.13$, without paternal age, Table S4).

In contrast to the high heritability of telomere length, the heritable component of the variation in telomere shortening was small with $h^2 \pm SE = 0.09 \pm 0.11$ (derived from the bivariate model, Table 1) and 0.08 ± 0.10 (derived from a univariate model with telomere shortening as dependent variable, Table S7).

Year was the only environmental factor that explained a significant part of the variance among individuals in early-life telomere length (age 4 days: 6%, Table 1, or 7%, $\chi^2 = 5.62$, $p = .018$, Table S3; age 29 days: 7%, Table 1, or 6%, $\chi^2 = 2.29$, $p = .13$, Table S6), indicating resemblance of telomere length between offspring within cohorts. Additional to the year effects present at the age of 4 days, there was a tendency for year effects leading to a significant variation in telomere shortening between the ages 4 and 29 days (ΔTL : 4%, $\chi^2 = 3.81$, $p = .05$, Table S7).

Early-life maternal effects, represented by biological mother ID, explained small nonsignificant fractions of the variance in telomere length at both ages (age 4 days: 4%, Table 1, or 4%, $\chi^2 = 0.53$, $p = .47$,

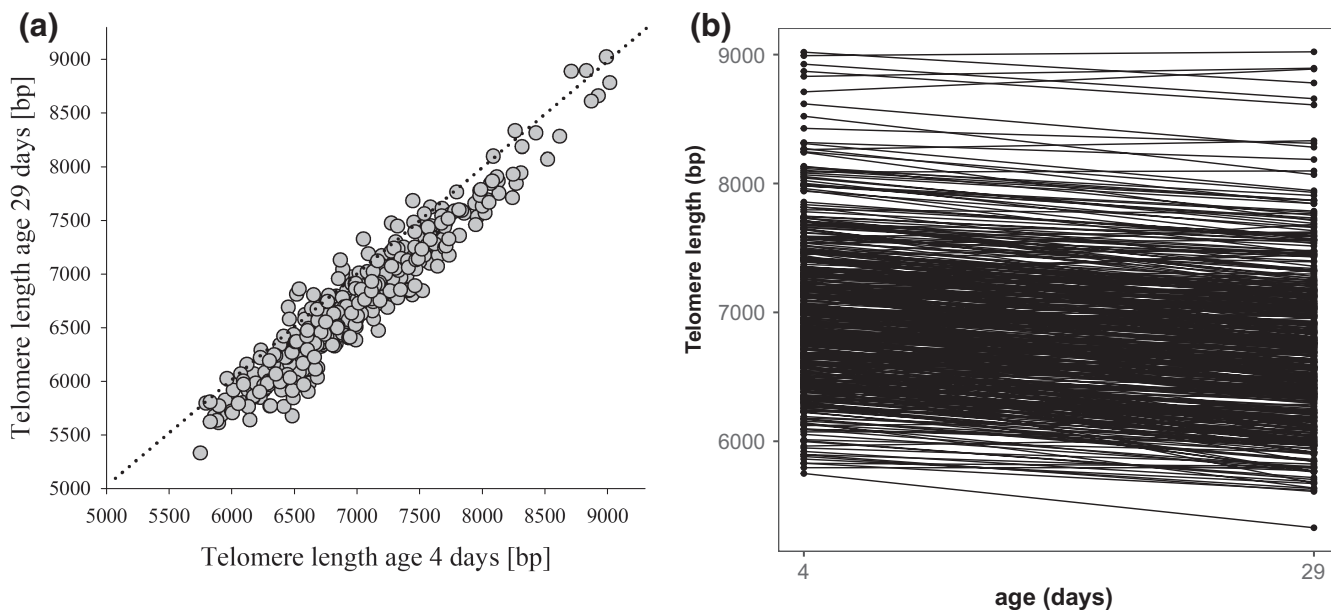


FIGURE 2 Repeated telomere length data within individuals ($n = 474$). (a) Telomere length at 29 days of age plotted against telomere length at 4 days of age in jackdaw nestlings. The dashed line represents $x = y$ and hence the perpendicular distance below this line reflects the telomere shortening. (b) Telomere length at the ages 4 and 29 days, where lines connect repeated data of the same individual

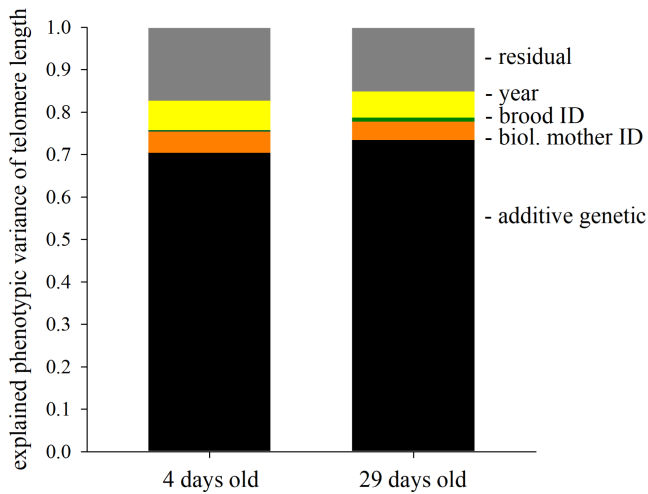


FIGURE 3 Components explaining phenotypic variance of telomere length among individuals measured at the ages 4 and 29 days derived from a bivariate animal model (Table 1). Components from bottom to top: additive genetic, biological mother ID, brood ID (note that it is hardly visible), year, residual

Table S3; age 29 days: 4%, Table 1, or bound to 0, Table S6). Biological father ID in the models was bound to zero (Table 1; Tables S3–S6). The mother that cared for the offspring from the age of 4 days onwards did not explain a noticeable amount of telomere length variation, and was bound to zero (Tables S6 and S7). The ID of the father that cared for the offspring was bound to zero in the model (Table S6) or explained a small, nonsignificant amount of variation in telomere shortening (ΔTL : 2%, $\chi^2 = 0.15$, $p = .70$, Table S7). Thus, telomere length of offspring cared for by the same parent, whether biological or nonbiological, did not show increased similarity due to parental care, neither when measured at the age of 4 days nor at the age of 29 days, or in their telomere shortening over this period.

Brood ID (pre- and post age 4 days, B1 and B2, respectively) explained little of the variation in telomere length at both sampling ages (age 4 days: B1: 0.3%, Table 1, or 5%, $\chi^2 = 1.39$, $p = .24$, Table S3; age 29 days: B1: bound to 0, Table S6, and B2: 0.6%, Table 1, or 2%, $\chi^2 = 0.45$, $p = .50$, Table S6; ΔTL : 2%, $\chi^2 = 0.09$, $p = .77$, Table S7). Thus, there was no significantly increased telomere length similarity of brood mates due to their shared brood environment, including brood-specific parental care effects.

TABLE 2 Parent–offspring regressions. Comparison of telomere length (TL) measured at the age of 4 days (in bp) of (a) mothers and their offspring ($n = 113$ individual offspring of 31 mothers) and (b) fathers and their offspring ($n = 111$ individual offspring of 28 fathers)

(a)					
Fixed effects	Estimate (SE)		F	df	p
Intercept	4,283.6 (781.6)				
Mother's TL (age 4 days)	0.418 (0.106)		15.44	1, 16.4	.001
Random effects	Variance (SE)	Proportion of variance (SE)	χ^2	df	p
Mother ID	71,974.6 (41,762.7)	0.205 (0.110)	3.940	1	.047
Brood ID (B1) ^a	–	–	–	–	–
Year	92,126.7 (66,701.9)	0.263 (0.148)	4.763	1	.029
Gel ID	14,888.4 (23,115.2)	0.042 (0.067)	0.521	1	.471
Residual	171,639.6 (31,312.4)	0.490 (0.125)			
(b)					
Fixed effects	Estimate (SE)		F	df	p
Intercept	6,865.4 (830.0)				
Father's TL (age 4 days)	0.102 (0.110)		0.861	1, 25.6	.362
Father's age at conception	–88.6 (38.2)		5.386	1, 20.1	.031
Random effects	Variance (SE)	Proportion of variance (SE)	χ^2	df	p
Father ID	62,097.5 (46,496.8)	0.200 (0.134)	1.948	1	.163
Brood ID (B1)	27,415.8 (45,317.5)	0.088 (0.147)	0.297	1	.586
Year	11,885.5 (22,898.4)	0.038 (0.072)	0.446	1	.504
Gel ID	7,893.1 (23,205.1)	0.025 (0.075)	0.134	1	.715
Residual	200,755.8 (40,829.6)	0.647 (0.137)			

^aBrood ID was not included in the final model as it was bound to 0.

Telomere lengths at the ages of 4 and 29 days were strongly genetically correlated ($r_G \pm SE = 0.999 \pm 0.006$, $p < .001$) and the additive genetic variances at the two ages (Table 1) did not differ ($p = .61$). Thus, there was no statistical support for a genotype by age interaction. The coefficients of additive genetic variance were 6.41% and 7.34% at ages of 4 and 29 days, respectively. Evolvability was 0.41% and 0.54% for telomere length at ages 4 and 29 days.

3.3 | Parent-offspring regressions

Telomere lengths of mothers and their offspring, all measured at the age of 4 days, were significantly positively related ($\beta = 0.42 \pm 0.11$), amounting to a telomere length heritability estimate of $h^2 = 0.84$ (Table 2a, Figure 4a). The same relationship for telomere lengths of fathers and their offspring was nonsignificant, whether paternal

age was included in the model ($\beta = 0.10 \pm 0.11$, Table 2b) or not ($\beta = 0.18 \pm 0.10$, Table S9; Figure 4b). Older fathers produced nestlings with shorter telomere length, as reflected in a significant negative paternal age effect. The slope of the mother-offspring regression was more than twice as steep as the slope derived from the father-offspring regression (Table 2). In contrast, paternal telomere length measured at the age of offspring conception was significantly positively related to offspring early-life telomere length ($\beta = 0.31 \pm 0.07$; Table S10), amounting to a telomere length heritability estimate of $h^2 = 0.61$.

Telomere shortening from age 4 to 29 days was neither significantly correlated between mother and offspring ($\beta = 0.18 \pm 0.24$), nor between father and offspring ($\beta = 0.04 \pm 0.17$; Table S11, Figure 4c,d). Thus, while being far from statistically significant, parent-offspring regressions yielded low estimates of the heritability of telomere shortening as the animal models (Table 1; Table S7).

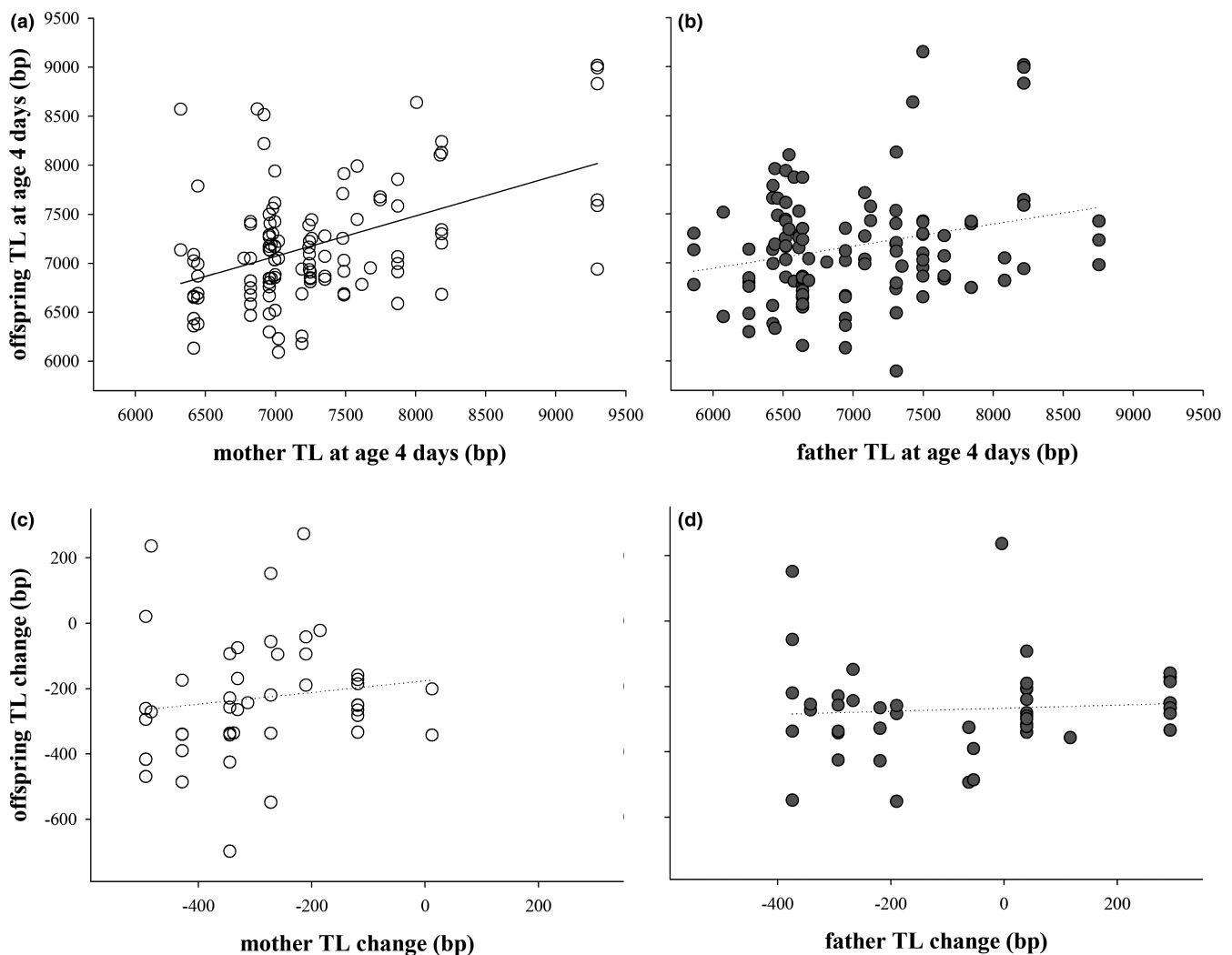


FIGURE 4 Parent-offspring regressions for telomere length and telomere shortening. Telomere length measured at the age of 4 days in (a) mothers ($n = 31$) and offspring ($n = 113$) and (b) fathers ($n = 28$) and offspring ($n = 111$). For statistics see Table 2. Change in telomere length between the ages of 4 and 29 days in (c) mothers ($n = 14$) and offspring ($n = 45$) and (d) fathers ($n = 12$) and offspring ($n = 45$). For statistics see Table S11. Open symbols for mother-offspring data points, filled symbols for father-offspring data points. Straight regression lines indicate a significant relationship, while dotted lines indicate a nonsignificant relationship

TABLE 3 Overview of the studies estimating heritability (h^2) of telomere length (TL) in nonhuman vertebrates

Species	Age TL measured	Cross-foster	Controlled environment ^b	Father age effect	Statistical analysis	h^2 (95% CI)	n	Cell type	Laboratory method	References
Birds										
King penguin <i>Aptenodytes patagonicus</i>	Offspring 10 days, parents at conception	No	No	No	Mid-parent-offspring mother-offspring	0.2 (-0.02 to 0.42) 0.2 (0.01–0.39)	53 53	Erythrocytes	qPCR	Reichert et al. (2015)
White-throated dipper <i>Cinclus cinclus</i>	7–17 days	No	Brood ID Cohort	No, but mother age effect	Mother-mid-offspring Father-mid-offspring Animal model	0.44 (0.048–0.83) 0.08 (-0.35 to 0.51) 0.038 (-0.10 to 0.17)	59 59 177	Erythrocytes	qPCR	Becker et al. (2015)
Great reed warbler <i>Acrocephalus arundinaceus</i>	8–10 days	No	Brood ID Mother ID	No, but mother age effect	Mother-mid-offspring Father-mid-offspring Animal model	1.08 n.s. 0.48 (0.24–0.72)	17 19 193	Erythrocytes	qPCR	Asghar et al., 2015
Collared flycatcher <i>Ficedula albicollis</i>	12 days	Yes	Brood triplets		Animal model (full siblings)	0.09 (-0.04 to 0.15)	359	Erythrocytes	qPCR	Voillenot et al. (2012)
Seychelles warbler <i>Acrocephalus sechellensis</i>	All ages	No	Mother ID Father ID Sample year Birth year Territory Permanent environment	Yes	Mother-offspring Father-offspring Animal model	n.s. n.s. 0.048 (<0.001–0.087) (2,664 samples)	303 284 1,318	Erythrocytes	qPCR	Sparks et al. (2021)
Kakapo <i>Strigops habroptilus</i>	1–35 years	No			Mother-offspring Father-offspring	0.84 n.s.	29 26	Erythrocytes	TRF ^c	Horn et al. (2011)
Tree swallow <i>Tachycineta bicolor</i>	Offspr. 12 days, parents at conception	Yes	Brood size	No	Mid-parent-offspring	0.81	122	Erythrocytes	TRF	Belmaker et al. (2019)
Zebra finch <i>Taeniopygia guttata</i>	9–636 days	Yes	Birth nest Rear nest Mother ID Father ID		Animal model	0.999 (0.87–1)	125	Erythrocytes	TRF	Atema et al. (2015)
Common tern <i>Sterna hirundo</i>	2–24 years	No	Permanent environment	Yes, not included	Animal model	0.63 (0.50–0.73)	387 (619 samples)	Erythrocytes	TRF	Vedder et al. (2021)
Jackdaw <i>Corvus monedula</i>	4 days* 29 days* Fathers at conception (ac)	Yes	Brood IDs Mother IDs Father IDs Birth year	Yes	Mother-offspring Father-offspring Father ac-offspring Animal model	0.84 (0.42–1) 0.20 (-0.23 to 0.63) 0.61 (0.32–0.90) 0.71 (0.47–0.95)* 0.77 (0.53–1.0)*	113 111 352 715 (1,189 samples)	Erythrocytes	TRF	This study
Reptiles										
Sand lizard <i>Lacerta agilis</i>	Hatchlings, known-age adults	No		Yes	Mother-daughter Father-son	0.52 (0.09–0.95) 1.23 (0.80–1.66)	55 40	Erythrocytes	TRF ^c	Olsson et al. (2011)
Fish										
Nine-spined sticklebacks <i>Pungitius pungitius</i>	122 days	Yes ^a	Temperature Family ID		Animal models (full siblings of 9 families)	0.31 (0.05–0.97) to 0.47 (0.17–0.91)	83; 213	Brain tissue	qPCR ^d	Noreikiene et al. (2017)

(Continues)

TABLE 3 (Continued)

Species	Age TL measured	Cross-foster	Controlled environment ^b	Father age effect	Statistical analysis	h^2 (95% CI)	<i>n</i>	Cell type	Laboratory method	References
Mammals										
Greater mouse-eared bat <i>Myotis myotis</i>	0–7+ years	No	Birth year Sample year Roost Temperature Rainfall		Animal models	0.01 (0.00–0.04) to 0.06 (0.02–0.11)	174 (504 samples)	Wing biopsy	qPCR	Foley et al. (2020)
Holstein Friesian dairy cattle	All at birth + later in life	No	Birth year Genetic group Permanent env.		Random regression model incl. pedigree information	0.36 to 0.47 (SE 0.05–0.08)	308 females (1328 samples)	Leucocytes	qPCR	Seeker et al. (2018)
Badger <i>Meles meles</i>	≤29 months * All ages *	No	Mother ID Father ID Birth year Sample year Social group Permanent env.	No	Animal model	<0.001 (<0.001–0.043) * <0.001 (>0.001–0.028) *	556 juveniles (837 samples) 612 (1,248 samples)	Leucocytes	qPCR	van Lieshout et al. (2020)
Soay sheep <i>Ovis aries</i>	4 months * > 1 year *	No	Mother ID Sample year Permanent env.	No	Animal model	0.29 (0.21–0.37) * 0.21 (0.16–0.26) *	1,582 (3,632 samples)	Leucocytes	qPCR	Froy et al. (2021)

*Reared individually after hatching.

^bRefers to animal models in studies with multiple statistical approaches.

^cTRF including interstitial telomeric sequences (Southern blot).

^dNo interstitial telomeric sequences in the genome.

^eHeritability estimated for different age classes.

4 | DISCUSSION

The main findings of our study were that telomere length was highly heritable in nestlings of free-living jackdaws, while heritable variation in telomere shortening was low. Telomeres shortened significantly and telomere lengths were phenotypically and genetically correlated within individuals. Nonetheless, as evidenced by the year effect, environmental influences can lead to significant variation in telomere length among individuals. Our finding that variation in telomere length and telomere shortening are primarily driven by additive genetic vs. environmental effects, respectively, suggests these traits differ in their potential as a fitness biomarker, and also in the mechanism through which this potential arises. Telomere length evolvability was low despite its high heritability.

Our estimate of telomere length heritability in jackdaws is similar to estimates reported by some other studies on free-living bird species such as terns and swallows (range $h^2 = 0.63\text{--}0.81$; Table 3), but substantially higher than estimates for penguins, dippers, warblers and flycatchers (range $h^2 = 0.0\text{--}0.48$; Table 3). One striking difference between those studies within the same taxonomic group (Aves) is that they used different telomere measurement techniques, TRF and qPCR, with higher and lower h^2 estimates respectively. Interestingly, TRF- and qPCR-based studies also report differences in telomere length repeatability, also being high and low, respectively (meta-analysis: Kärkkäinen et al., 2021). This is of relevance as phenotypic trait repeatability usually sets the upper limit to heritability (Dohm, 2002). The difference in heritability and repeatability estimates between TRF- and qPCR-based studies may be due to chance, given that the number of studies is still small, but may also be caused by real differences between the methods. With respect to the latter possibility, two main differences between TRF and qPCR measurements that may contribute to variation in repeatability and heritability estimates are that: (i) qPCR-based measurements include all telomeric sequences within the genome, and thus terminally and interstitially located telomeric sequences, which are excluded when applying TRF to non-denatured DNA, whereas TRF-based measurements include a potentially variable subtelomeric region (Baird, 2005; Nussey et al., 2014); and (ii) measurement reliability tends to be lower when using qPCR (Horn et al., 2010; Morinha et al., 2020; Nussey et al., 2014). Regarding the potential influence of interstitial telomeric sequences on telomere length, their abundance in length and number varies between and within species (Delany et al., 2000; Foote et al., 2013; Meyne et al., 1990; Olsson et al., 2011). However, for the inclusion of interstitial telomeric sequences to explain the lower repeatability and heritability estimates when using qPCR, interstitial telomeric sequences would have to be more susceptible to environmental effects than terminal telomeres, resulting in a higher absolute change in the total number of telomere sequence repeats in the genome. There is at present little indication that this is a likely scenario. With respect to the subtelomeric region included in the TRF measurements, multiple tests on the extent of subtelomeric region measured, including a comparison between results from the two measurement techniques by Atema et al. (2019), suggest a minor

influence on absolute telomere length, depending on the set of restriction enzymes used. We therefore consider, and also given the findings of Kärkkäinen et al. (2021), that measurement error, introduced at any point between sample collection and final estimation, is the most parsimonious explanation for the difference in repeatability and heritability estimates between qPCR- and TRF-based studies.

Heritability estimates for telomere length were high at both ages, and environmental effects had a relatively small influence on telomere length (Table 1; Tables S3–S8). Consequently, heritability estimates were similar whether derived from “animal model” analyses or parent–offspring regressions or whether shared environmental effects were controlled for in the models. The high phenotypic correlation of telomere length early and late in the nestling period, when telomere shortening is higher than later in life (Boonekamp et al., 2014; Salomons et al., 2009), and the consequently similarly high heritability estimates for both ages suggest that heritability of telomere length is high independent of age in our population, at least within the narrow age range over which we can make this comparison.

Among the tested environmental components, year effects explained a significant but small part of the variation in telomere length in our study population (Table 1; Tables S3 and S7). Birth cohort effects on telomere length have also been identified in other free-living vertebrates, including white-throated dippers (*Cinclus cinclus*) (Becker et al., 2015) and Soay sheep (*Ovis aries*) (Fairlie et al., 2016). However, the proportion of telomere length variance explained by year differed strongly between studies (6% in our study vs. 46% in dippers or nonsignificant in Seychelles warblers, Sparks et al., 2021). The cause of variation in telomere length between cohorts in our study population, already present at the age of 4 days, remains to be identified. Experimental studies found temperature conditions during incubation (Vedder et al., 2018) and glucocorticoid concentrations in the eggs (Hausmann et al., 2012) to affect offspring telomere length at this early stage in life, but whether these factors also modulate telomere length generally or depending on year remains to be established. The variation in telomere shortening between years that we found from the age of 4 days onwards can arise in combination with brood size, as shown previously in this population (Boonekamp et al., 2014).

To disentangle potential early-life parental effects on nestling telomere length, via egg content or parental care, we cross-fostered clutches and part of the offspring to nongenetic parents. Our results show that early-life parental effects did not increase telomere length similarity between nestlings (genetic and nongenetic siblings), as also reported for dippers (Becker et al., 2015) and Seychelles warblers (Sparks et al., 2021), but unlike reed warblers (Ashgar et al., 2015). Our results also do not support that a shared brood environment increased telomere length similarity of nest mates at any age, while this has been found, for example, in dippers (Becker et al., 2015).

Parent–offspring correlations of early-life telomere length were stronger for mothers than for fathers. This could result either from a stronger maternal inheritance of telomere length or from early-life effects affecting telomere length similarly in mother and offspring.

Alternatively, it may reflect that gamete telomere length changes more over life in fathers than in mothers, given that all oocytes are already present at birth while sperm is formed throughout life (Bauch et al., 2019). Early-life maternal effects were negligible according to our animal model results and are therefore unlikely to explain the higher similarity between mother and offspring early-life telomere length. However, we found a significantly positive correlation between father telomere length at the age of offspring conception and offspring telomere length at the age of 4 days. This suggests an epigenetic effect via declining telomere length in ageing fathers that is transferred to offspring via sperm (Bauch et al., 2019; Eisenberg, 2019; Sparks et al., 2021). Thus, taking into account paternal epigenetic inheritance, our results support quantitatively similar maternal and paternal contributions to telomere length inheritance.

We estimated the heritable component in the amount of telomere shortening early in life to be small (Table 1; Tables S7 and S11). The only other study of which we are aware that estimated heritability of telomere shortening, based on adult human twins, found a moderately low heritability ($h^2 = 0.28$; Hjelmborg et al., 2015). Telomere length early and late in the nestling period showed a strong genetic correlation between both ages ($r_G = .999$), thus supporting that telomere length at both ages is controlled by the same genes. This is in line with findings in other wild vertebrates later in life (Froy et al., 2021; Vedder et al., 2021). It should be noted, however, that the number of base pairs telomeres shortened over the nestling period (mean \pm SD = 260 \pm 171) is small relative to the variation in telomere length at any age (SD = 590), potentially masking heritable variation in telomere shortening.

Fisher (1930) predicted that heritability of traits declines with their effect on fitness (a prediction that was empirically broadly confirmed; e.g., Mousseau & Roff, 1987). Our finding that additive genetic effects explained most variation in telomere length, but little variation in telomere shortening, fits this pattern, because previous investigations in our population showed that telomere shortening was a better predictor of survival than absolute telomere length (Boonekamp et al., 2014; Salomons et al., 2009).

It is tempting to suppose that a high heritability indicates that directional selection on telomere length could produce a fast evolutionary response. However, the high heritability estimate, representing the ratio between additive genetic and total phenotypic variance, results from the low environmental sensitivity that we find in our population in combination with a low amount of additive genetic variance (scaled to the mean: CV = 6.41% or 7.34%). The low additive genetic variance related to trait size resulted in a low evolvability estimate of telomere length. Telomere length evolvability was of similar magnitude in field crickets (Boonekamp et al., 2021). Whether this should be considered low or high is not obvious, but our evolvability estimate of 0.41% at age 4 days would allow an increase by 29 bp per generation, or close to 20 generations to increase population telomere length by 1 SD. More studies that provide evolvability estimates for comparison among populations and taxa and investigate the genetic link between telomere length and fitness are required to gain a more comprehensive picture.

ACKNOWLEDGEMENTS

We thank Martijn Salomons, the late Cor Dijkstra and the many helpers involved in data and blood sample collection in this long-term project, Erica Zuidersma, who contributed to the telomere analyses, Alastair Wilson for advice on the bivariate model analysis and three reviewers for valuable comments on the manuscript. C.B. was supported by a DFG research fellowship (BA 5422/1-1) and J.J.B. by an NWO grant (823.01.009) awarded to S.V.

AUTHOR CONTRIBUTIONS

S.V. designed and organized the long-term project; J.J.B., S.V. and C.B. carried out fieldwork; E.M. and C.B. carried out laboratory work; C.B., J.J.B. and P.K. managed and analysed data; C.B. led the writing of the manuscript and all authors were involved in the reviewing and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.k6djh9w74> (Bauch et al., 2021).

ORCID

Christina Bauch  <https://orcid.org/0000-0003-0218-5582>

Jelle J. Boonekamp  <https://orcid.org/0000-0003-1900-627X>

Peter Korsten  <https://orcid.org/0000-0003-0814-9099>

Ellis Mulder  <https://orcid.org/0000-0002-1956-8734>

Simon Verhulst  <https://orcid.org/0000-0002-1143-6868>

REFERENCES

- Angelier, F., Costantini, D., Blévin, P., & Chastel, O. (2018). Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. *General and Comparative Endocrinology*, 256, 99–111. <https://doi.org/10.1016/j.ygcen.2017.07.007>
- Asghar, M., Bensch, S., Tarka, M., Hansson, B., & Hasselquist, D. (2015). Maternal and genetic factors determine early life telomere length. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20142263. <https://doi.org/10.1098/rspb.2014.2263>
- Atema, E., Mulder, E., Dugdale, H. L., Briga, M., van Noordwijk, A. J., & Verhulst, S. (2015). Heritability of telomere length in the Zebra Finch. *Journal of Ornithology*, 156, 1113–1123. <https://doi.org/10.1007/s10336-015-1212-7>
- Atema, E., Mulder, E., van Noordwijk, A. J., & Verhulst, S. (2019). Ultralong telomeres shorten with age in nestling great tits but are static in adults and mask attrition of short telomeres. *Molecular Ecology Resources*, 19, 648–658. <https://doi.org/10.1111/1755-0998.12996>
- Baird, D. M. (2005). New developments in telomere length analysis. *Experimental Gerontology*, 40, 363–368. <https://doi.org/10.1016/j.exger.2005.02.008>
- Barrett, E. L. B., Burke, T. A., Hammers, M., Komdeur, J., & Richardson, D. S. (2013). Telomere length and dynamics predict mortality in a wild longitudinal study. *Molecular Ecology*, 22, 249–259. <https://doi.org/10.1111/mec.12110>
- Barrett, E. L. B., & Richardson, D. S. (2011). Sex differences in telomeres and lifespan. *Aging Cell*, 10, 913–921. <https://doi.org/10.1111/j.1474-9726.2011.00741.x>

- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2019). Epigenetic inheritance of telomere length in wild birds. *PLoS Genetics*, 15, e1007827. <https://doi.org/10.1371/journal.pgen.1007827>
- Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2021). Data from: High heritability of telomere length and low heritability of telomere shortening in wild birds. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.k6djh9w74>
- Bauch, C., Gatt, M. C., Granadeiro, J. P., Verhulst, S., & Catry, P. (2020). Sex-specific telomere length and dynamics in relation to age and reproductive success in Cory's shearwaters. *Molecular Ecology*, 29, 1344–1357. <https://doi.org/10.1111/mec.15399>
- Becker, P. J. J., Reichert, S., Zahn, S., Hegelbach, J., Masseurin, S., Keller, L. F., Postma, E., & Criscuolo, F. (2015). Mother-offspring and nest-mate resemblance but no heritability in early-life telomere length in white-throated dippers. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20142924. <https://doi.org/10.1098/rspb.2014.2924>
- Belmaker, A., Hallinger, K. K., Glynn, R. A., Winkler, D. W., & Haussmann, M. F. (2019). The environmental and genetic determinants of chick telomere length in Tree Swallows (*Tachycineta bicolor*). *Ecology and Evolution*, 9, 8175–8186. <https://doi.org/10.1002/ece3.5386>
- Benetos, A., Verhulst, S., Labat, C., Lai, T.-P., Girerd, N., Toupance, S., Zannad, F., Rossignol, P., & Aviv, A. (2019). Telomere length tracking in children and their parents: implications for adult onset diseases. *The FASEB Journal*, 33, 14248–14253. <https://doi.org/10.1096/fj.201901275R>
- Blackburn, E. H. (1991). Structure and function of telomeres. *Nature*, 350, 569–573. <https://doi.org/10.1146/annurev.genet.23.1.579>
- Boonekamp, J. J., Bauch, C., & Verhulst, S. (2020). Experimentally increased brood size accelerates actuarial senescence and increases subsequent reproductive effort in a wild bird population. *Journal of Animal Ecology*, 89, 1395–1407. <https://doi.org/10.1111/1365-2656.13186>
- Boonekamp, J. J., Mulder, G. A., Salomons, H. M., Dijkstra, C., & Verhulst, S. (2014). Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20133287. <https://doi.org/10.1098/rspb.2013.3287>
- Boonekamp, J. J., Rodriguez-Munoz, R., Hopwood, P., Zuidersma, E., Mulder, E., Wilson, A., & Tregenza, T. (2021). Telomere length is highly heritable and independent of growth rate manipulated by temperature in field crickets. *Molecular Ecology*, <https://doi.org/10.1111/mec.15888>
- Boonekamp, J. J., Simons, M. J. P., Hemerik, L., & Verhulst, S. (2013). Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging Cell*, 12, 330–332. <https://doi.org/10.1111/accel.12050>
- Broer, L., Codd, V., Nyholt, D. R., Deelen, J., Mangino, M., Willemsen, G., Albrecht, E., Amin, N., Beekman, M., de Geus, E. J. C., Henders, A., Nelson, C. P., Steves, C. J., Wright, M. J., de Craen, A. J. M., Isaacs, A., Matthews, M., Moayyeri, A., Montgomery, G. W., ... Boomsma, D. I. (2013). Meta-analysis of telomere length in 19 713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *European Journal of Human Genetics*, 21, 1163–1168. <https://doi.org/10.1038/ejhg.2012.303>
- Chan, S. R. W. L., & Blackburn, E. H. (2004). Telomeres and Telomerase. *Philosophical Transactions of the Royal Society London B*, 359, 109–121. <https://doi.org/10.1098/rstb.2003.1370>
- Codd, V., Nelson, C. P., Albrecht, E., Mangino, M., Deelen, J., Buxton, J. L., Hottenga, J. J., Fischer, K., Esko, T., Surakka, I., Broer, L., Nyholt, D. R., Leach, I. M., Salo, P., Hägg, S., Matthews, M. K., Palmen, J., Norata, G. D., O'Reilly, P. F., ... Samani, N. J. (2013). Identification of seven loci affecting mean telomere length and their association with disease. *Nature Genetics*, 45, 422–427. <https://doi.org/10.1038/ng.2528>
- Criscuolo, F., Zahn, S., & Bize, P. (2017). Offspring telomere length in the long lived Alpine swift is negatively related to the age of their biological father and foster mother. *Biology Letters*, 13, 20170188. <https://doi.org/10.1098/rsbl.2017.0188>
- de Lange, T. (2005). Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes & Development*, 19, 2100–2110. <https://doi.org/10.1101/gad.1346005>
- Delany, M. E., Krupkin, A. B., & Miller, M. M. (2000). Organization of telomere sequences in birds: evidence for arrays of extreme length and for in vivo shortening. *Cytogenetics and Cell Genetics*, 90, 139–145. <https://doi.org/10.1159/000015649>
- Dohm, M. R. (2002). Repeatability estimates do not always set an upper limit to heritability. *Functional Ecology*, 16, 273–280.
- Dugdale, H. L., & Richardson, D. S. (2018). Heritability of telomere variation: it is all about the environment!. *Philosophical Transactions of the Royal Society B*, 373, 20160450. <https://doi.org/10.1098/rstb.2016.0450>
- Eisenberg, D. T. A. (2019). Paternal age at conception effects on offspring telomere length across species – What explains the variability? *PLoS Genetics*, 15, e1007946. <https://doi.org/10.1371/journal.pgen.1007946>
- Fairlie, J., Holland, R., Pilkington, J. G., Pemberton, J. M., Harrington, L., & Nussey, D. H. (2016). Lifelong leukocyte telomere dynamics and survival in a free-living mammal. *Aging Cell*, 15, 140–148. <https://doi.org/10.1111/accel.12417>
- Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics*, 4th ed. .
- Fisher, R. A. (1930). *The genetical theory of natural selection*. Dover.
- Foley, N., Petit, E. J., Brazier, T., Finarelli, J. A., Hughes, G. M., Touzalin, F., & Teeling, E. C. (2020). Drivers of longitudinal telomere dynamics in a long-lived bat species, *Myotis myotis*. *Molecular Ecology*, 29, 2963–2977. <https://doi.org/10.1111/mec.15395>
- Foote, C. G., Vleck, D., & Vleck, C. M. (2013). Extent and variability of interstitial telomeric sequences and their effects on estimates of telomere length. *Molecular Ecology Resources*, 13, 417–428. <https://doi.org/10.1111/1755-0998.12079>
- Froy, H., Underwood, S. L., Dorrens, J., Seeker, L. A., Watt, K., Wilbourn, R. V., Nussey, D. H. (2021) Heritable variation in telomere length predicts mortality in Soay sheep. *Proceedings of the National Academy of Sciences of the USA*, 118, e2020563118.
- Griffiths, R., Double, M. C., Orr, K., & Dawson, R. J. G. (1998). A DNA test to sex most birds. *Molecular Ecology*, 7, 1071–1075. <https://doi.org/10.1046/j.1365-294x.1998.00389.x>
- Haussmann, M. F., Longenecker, A. S., Marchetto, N. M., Juliano, S. A., & Bowden, R. M. (2012). Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere length. *Proceedings of the Royal Society of London B: Biological Sciences*, 279, 1447–1456. <https://doi.org/10.1098/rspb.2011.1913>
- Henderson, I. G., Hart, P. J. B., & Burke, T. (2000). Strict monogamy in a semi-colonial passerine: the Jackdaw *Corvus monedula*. *Journal of Avian Biology*, 31, 177–182. <https://doi.org/10.1034/j.1600-048X.2000.310209.x>
- Hjelmborg, J. B., Dalgård, C., Möller, S., Steenstrup, T., Kimura, M., Christensen, K., Kyvik, K. O., & Aviv, A. (2015). The heritability of leukocyte telomere length dynamics. *Journal of Medical Genetics*, 52, 297–302. <https://doi.org/10.1136/jmedgenet-2014-102736>
- Horn, T., Robertson, B. C., & Gemmill, N. J. (2010). The use of telomere length in ecology and evolutionary biology. *Heredity*, 105, 497–506. <https://doi.org/10.1038/hdy.2010.113>
- Horn, T., Robertson, B. C., Will, M., Eason, D. K., Elliott, G. P., & Gemmill, N. J. (2011). Inheritance of telomere length in a bird. *PLoS One*, 6, e17199. <https://doi.org/10.1371/journal.pone.0017199>

- Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics*, 130, 195–204. <https://doi.org/10.1093/genetics/130.1.195>
- Kärkkäinen, T., Briga, M., Laaksonen, T., Stier, A., Kärkkäinen, Tiia, Briga, Michael, Laaksonen, Toni & Stier, Antoine (2021). Within-individual repeatability in telomere length: a meta-analysis in non-mammalian vertebrates. *Molecular Ecology*. <http://dx.doi.org/10.1111/mec.16155>
- Kotschal, A., Ilmonen, P., & Penn, D. J. (2007). Stress impacts telomere dynamics. *Biology Letters*, 3, 128–130. <https://doi.org/10.1098/rsbl.2006.0594>
- Kruuk, L. E. B., & Hadfield, J. D. (2007). How to separate genetic and environmental causes of similarity between relatives. *Journal of Evolutionary Biology*, 20, 1890–1903. <https://doi.org/10.1111/j.1420-9101.2007.01377.x>
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017) lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82, 1–26.
- Liebers, D., & Peter, H.-U. (1998). Intraspecific interactions in jackdaws *Corvus monedula*: A field study combined with parentage analysis. *Ardea*, 86, 221–235.
- Lieshout, S. H. J., Sparks, A. M., Bretman, A., Newman, C., Buesching, C. D., Burke, T., Macdonald, D. W., & Dugdale, H. L. (2020). Estimation of environmental, genetic and parental age at conception effects on telomere length in a wild mammal. *Journal of Evolutionary Biology*, 34(2), 296–308. <https://doi.org/10.1111/JEB.13728>
- Marasco, V., Boner, W., Griffiths, K., Heidinger, B., & Monaghan, P. (2019). Intergenerational effects on offspring telomere length: interactions among maternal age, stress exposure and offspring sex. *Proceedings of the Royal Society of London B: Biological Sciences*, 286, 20191845. <https://doi.org/10.1098/rspb.2019.1845>
- McLennan, D., Armstrong, J. D., Stewart, D. C., McKelvey, S., Boner, W., Monaghan, P., & Metcalfe, N. M. (2016). Interactions between parental traits, environmental harshness and growth rate in determining telomere length in wild juvenile salmon. *Molecular Ecology*, 25, 5425–5438. <https://doi.org/10.1111/mec.13857>
- Meyne, J., Baker, R. J., Hobart, H. H., Hsu, T. C., Ryder, O. A., Ward, O. G., Wiley, J. E., Wurster-Hill, D. H., Yates, T. L., & Moyzis, R. K. (1990). Distribution of non-telomeric sites of the (TTAGGG)_n telomeric sequence in vertebrate chromosomes. *Chromosoma*, 99, 3–10. <https://doi.org/10.1007/BF01737283>
- Monaghan, P., Eisenberg, D. T. A., Harrington, L., & Nussey, D. H. (Eds.). (2018) Understanding diversity in telomere dynamics [Special issue]. *Philosophical Transactions of the Royal Society B Biological Sciences*, 373, 1741.
- Morinha, F., Magalhães, P., & Blanco, G. (2020). Standard guidelines for the publication of telomere qPCR results in evolutionary ecology. *Molecular Ecology Resources*, 20, 635–648. <https://doi.org/10.1111/1755-0998.13152>
- Morrissey, M. B., & Wilson, A. J. (2010). PEDANTICS: an R package for pedigree-based genetic simulation and pedigree manipulation, characterization and viewing. *Molecular Ecology Resources*, 10, 711–719. <https://doi.org/10.1111/j.1755-0998.2009.02817.x>
- Mousseau, T., & Roff, D. (1987). Natural selection and the heritability of fitness components. *Heredity*, 59, 181–197. <https://doi.org/10.1038/hdy.1987.113>
- Noguera, J. C., Metcalfe, N. B., & Monaghan, P. (2018). Experimental demonstration that offspring fathered by old males have shorter telomeres and reduced lifespans. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20180268. <https://doi.org/10.1098/rspb.2018.0268>
- Noreikiene, K., Kuparinen, A., & Merilä, J. (2017). Age at maturation has sex- and temperature-specific effects on telomere length in a fish. *Oecologia*, 184, 767–777. <https://doi.org/10.1007/s00442-017-3913-5>
- Nussey, D. H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N., Hartmann, N., Horn, T., Haussmann, M., Olsson, M., Turbill, C., Verhulst, S., Zahn, S., & Monaghan, P. (2014). Measuring telomere length and telomere dynamics in evolutionary biology and ecology. *Methods in Ecology and Evolution*, 5, 299–310. <https://doi.org/10.1111/2041-210X.12161>
- O'Sullivan, R. J., & Karlseder, J. (2010). Telomeres: protecting chromosomes against genome instability. *Nature Reviews: Molecular Cell Biology*, 11, 171–181. <https://doi.org/10.1038/nrm2848>
- Olsson, M., Pauliny, A., Wapstra, E., Uller, T., Schwartz, T., & Blomqvist, D. (2011). Sex differences in sand lizard telomere inheritance: paternal epigenetic effects increases telomere heritability and offspring survival. *PLoS One*, 6, e17473. <https://doi.org/10.1371/journal.pone.0017473>
- R Core Team (2017). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Reichert, S., Rojas, E. R., Zahn, S., Robin, J. P., Criscuolo, F., & Massemin, S. (2015). Maternal telomere length inheritance in the king penguin. *Heredity*, 114, 10–16. <https://doi.org/10.1038/hdy.2014.60>
- Röell, A. (1978). Social behaviour of the jackdaw, *Corvus monedula*, in relation to its niche. *Behaviour*, 64, 1–124.
- Salomons, H. M., Mulder, G. A., van de Zande, L., Haussmann, M. F., Linskens, M. H. K., & Verhulst, S. (2009). Telomere shortening and survival in free-living corvids. *Proceedings of the Royal Society of London B: Biological Sciences*, 276, 3157–3165. <https://doi.org/10.1098/rspb.2009.0517>
- Seeker, L. A., Ilska, J. J., Psifidi, A., Wilbourn, R. V., Underwood, S. L., Fairlie, J., Holland, R., Froy, H., Bagnall, A., Whitelaw, B., Coffey, M., Nussey, D. H., & Banos, G. (2018). Longitudinal changes in telomere length and associated genetic parameters in dairy cattle analysed using random regression models. *PLoS ONE*, 13(2), e0192864. <https://doi.org/10.1371/journal.pone.0192864>
- Seeker, L. A., Underwood, S. L., Wilbourn, R. V., Dorrens, J., Froy, H., Holland, R., Ilska, J. J., Psifidi, A., Bagnall, A., Whitelaw, B., Coffey, M., Banos, G., & Nussey, D. H. (2021). Telomere attrition rates are associated with weather conditions and predict productive lifespan in dairy cattle. *Scientific Reports*, 11, 5589. <https://doi.org/10.1038/s41598-021-84984-2>
- Sheldon, E., Eastwood, J., Teunissen, N., Roast, M., Fan, M., Hall, M., & Peters, A. (2021). Telomere dynamics in the first year of life, but not later in life, predict lifespan in a wild bird. *Authorea Preprints*, <https://doi.org/10.22541/au.162168440.03821228/v1>
- Soerensen, M., Thinggaard, M., Nygaard, M., Dato, S., Tan, Q., Hjelmborg, J., & Christiansen, L. (2012). Genetic variation in TERT and TERC and human leukocyte telomere length and longevity: a cross-sectional and longitudinal analysis. *Aging Cell*, 11, 223–227. <https://doi.org/10.1111/j.1474-9726.2011.00775.x>
- Sparks, A. M., Spurgin, L. G., van der Velde, M., Fairfield, E. A., Komdeur, J., Burke, T., & Dugdale, H. L. (2021). Telomere heritability and parental age at conception effects in a wild avian population. *Molecular Ecology*, <https://doi.org/10.1111/mec.15804>
- Spurgin, L. G., Bebbington, K., Fairfield, E. A., Hammers, M., Komdeur, J., Burke, T., Dugdale, H. L., & Richardson, D. S. (2018). Spatio-temporal variation in lifelong telomere dynamics in a long-term ecological study. *Journal of Animal Ecology*, 87, 187–198. <https://doi.org/10.1111/1365-2656.12741>
- Vedder, O., Moiron, M., Bichet, C., Bauch, C., Verhulst, S., Becker, P. H., & Bouwhuis, S. (2021). Telomere length is heritable and genetically correlated with lifespan in a wild bird. *Molecular Ecology*, <https://doi.org/10.1111/mec.15807>
- Vedder, O., Verhulst, S., Zuiderma, E., & Bouwhuis, S. (2018). Embryonic growth rate affects telomere attrition: an experiment in a wild bird. *Journal of Experimental Biology*, 221, jeb181586. <https://doi.org/10.1242/jeb.181586>
- Voillemot, M., Hine, K., Zahn, S., Criscuolo, F., Gustafsson, L., Doligez, B., & Bize, P. (2012). Effects of brood size manipulation and common origin

- on phenotype and telomere length in nestling collared flycatchers. *BMC Ecology*, 12, 17. <https://doi.org/10.1186/1472-6785-12-17>
- Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H., & Boonekamp, J. J. (2018). The relationship between telomere length and mortality risk in non-model vertebrate systems: a meta-analysis. *Philosophical Transactions of the Royal Society B*, 373, 20160447. <https://doi.org/10.1098/rstb.2016.0447>
- Wilson, A. J. (2008). Why h^2 does not always equal V_A/V_P ? *Journal of Evolutionary Biology*, 21, 647–650. <https://doi.org/10.1111/j.1420-9101.2008.01500.x>
- Wilson, A. J., Réale, D., Clements, M. N., Morrissey, M. M., Postma, E., Walling, C. A., Kruuk, L. E. B., & Nussey, D. H. (2010). An ecologist's guide to the animal model. *Journal of Animal Ecology*, 79, 13–26. <https://doi.org/10.1111/j.1365-2656.2009.01639.x>
- Young, A. (2018). The role of telomeres in the mechanisms and evolution of life-history trade-offs and ageing. *Philosophical Transactions of the Royal Society B Biological Sciences*, 373, 20160452. <https://doi.org/10.1098/rstb.2016.0452>

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., & Verhulst, S. (2022). High heritability of telomere length and low heritability of telomere shortening in wild birds. *Molecular Ecology*, 31, 6308–6323. <https://doi.org/10.1111/mec.16183>