Heritability and Parental Effects in Telomere Length in a Color Polymorphic Long-Lived Bird

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ABSTRACT

Relative telomere length (RTL), an indicator of senescence, has been shown to be heritable but can also be affected by environmental factors, such as parental effects. Investigating heritability as well as parental effects and rearing environment can help us to understand the factors affecting offspring telomeres. Moreover, how phenotypic parental traits linked with fitness can impact offspring RTL is still unclear. A phenotypic marker closely associated with physiological traits and fitness is melanin-based color polymorphism, which in tawny owl (*Strix aluco*) is highly heritable and strongly associated with adult telomere shortening and survival. We studied narrow-sense heritability (h^2) of RTL, as well as the impact of parental age and color morph and their interaction on offspring RTL. Offspring RTL at fledging was strongly positively correlated with both mother RTL and father RTL at breeding. Offspring RTL was also negatively associated with father age, suggesting that older fathers sired offspring with shorter telomeres. Parental color morph did not explain offspring RTL, and there were no interactive effects of parental morph and age, despite previously documented morph-specific senescence patterns. Our results suggest that RTL is highly heritable and affected by paternal age but not related to color polymorphism. This suggests that either morph-specific telomere shortening as an adult does not result in significantly shorter telomeres in their gametes, or that parents compensate morph-specific senescence via parental care. Morph-specific patterns of telomere dynamics in polymorphic species may thus emerge from different life history strategies adopted in adulthood.

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Introduction

Parents may affect offspring survival and phenotype not only through genetic factors but also via parental effects, which can occur at different stages from gamete formation to offspring development (Perez and Lehner 2019). Intergenerational effects (genetic, epigenetic, or parental effects) can thus affect offspring phenotype, physiology, and behavior. Understanding how physiological traits are affected by the parental phenotype, and how they are inherited across generations, is important to understand the proximate mechanisms underlying life history strategies. A key physiological marker that could help disentangle the links between parental effects, physiological traits, and life history strategy is relative telomere length (RTL). Indeed, telomere dynamics have been linked to survival and senescence and may play a role in the evolution of life history trade-offs (Monaghan and Haussmann 2006; Haussmann and Marchetto 2010; Monaghan et al. 2010; Angelier et al. 2018).

Telomeres are noncoding, highly structured repeated DNA sequences located at the ends of eukaryotic chromosomes, and their length is a predictor of individual life expectancy and fitness (Bize et al. 2009; Heidinger et al. 2012). Telomere dynamics could thus be a strong indicator of individual fitness, in terms of both reproduction and survival (Wilbourn et al. 2018; Angelier et al. 2019), and of the quality of parental care (Viblanc et al. 2020). The RTL of an individual at a certain life stage will depend on the RTL at birth and on the trade-off between the rate of telomere loss across a lifetime and the rate of telomere restoration (via the action of, e.g., the enzyme telomerase; Monaghan 2014; Monaghan and Ozanne 2018). Telomere loss occurs naturally during a lifetime because of accumulated damage during each DNA replication but also in response to environmental and physiological sources of stress (Monaghan et al. 2010; Monaghan 2014; Angelier et al. 2018; Chatelain et al. 2020). Telomeres are known to be susceptible to oxidative stress (Haussmann and Marchetto 2010; Angelier et al. 2018; but see Reichert and Stier 2017) and are biomarkers of aging.

In birds, RTL of all age classes presents high individual variation. Parts of this variation have been found to be due

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to inheritance from parents, but the observed inheritance patterns are complex and differ substantially in magnitude (table 1). This inconsistency is partly due to variation in study design and statistical tools to make these estimates (Dugdale and Richardson 2018). In addition to being heritable, offspring RTL is also strongly affected by environmental and parental effects (e.g., Angelier et al. 2018; Dugdale and Richardson 2018; Bauch et al. 2019). If the aim is to estimate the genetic versus environmental components (e.g., nongenetic parental effects, cohort effects, and variation in food availability among territories; Dugdale and Richardson 2018) of RTL, it is important to have large data sets that allow statistical separation of these effects in a quantitative genetic framework (Dugdale and Richardson 2018).

Parental traits, such as age, can also strongly impact offspring phenotype. The impact of parental age on offspring longevity is called the Lansing effect (review in Monaghan et al. 2020), and evidence is accumulating that offspring telomere dynamics are

Table 1: Summary of published literature on telomere heritability (h^2) in avian species

Source	Species	Exp	Sex role	Father-offspring (h^2)	Mother-offspring (h^2)	Parent-offspring h^2 (h^2 animal model)	Parental sample	Method
				Maternal				
Horn et al. 2011[a]	Kakapo	No	A	NA	.84	NA (NA)	Adults	TRF
Asghar et al. 2015	Great reed warbler	No	B	.28	1.08	NA (.48)	Nestlings	qPCR
Reichert et al. 2015	King penguin	No	B	NA	.2	.2 (NA)	Breeding	qPCR
Becker et al. 2015	White-throated dipper	No	B	.08	.44	.44 (.038)	Nestlings	qPCR
Öst et al. 2020[b]	Eider duck	No	A	NA	NA	NA (NA)	Breeding	qPCR
				Both parents				
Belmaker et al. 2019	Tree swallow	Yes	B	NA	NA	.81 (NA)	Breeding	TRF
Bauch et al. 2019	Jackdaws	Yes	B	NA	NA	.72 (NA)	Breeding	TRF
Vedder et al. 2021[c]	Common tern	No	B	NA	NA	NA (.63)	Adults	TRF
Sparks et al. 2021	Seychelles warbler	No	B	NA	NA	NA (.048)	Adults	qPCR
This study	Tawny owl	No	C	1.04	.82	.48 (.32)	Breeding	qPCR
				Relation with cross-foster parents				
Viblanc et al. 2020[d]	King penguin	Yes	B	NA	NA	NA (NA)	Breeding	qPCR
				Full/half-sibling				
Voillemot et al. 2012[e]	Collared flycatcher	Yes	B	NA	NA	NA (.09)	No samples	qPCR
Atema et al. 2015[f]	Zebra finch	Yes	B	.93	1.35	NA (.999)	No samples	TRF

Note. Experimental manipulation (exp; i.e., cross-fostering) is marked as yes/no. "Sex roles" refers to sex roles in parental care classified as A for only female care, B for both parents providing equal parental care, and C for distinct sex roles in parental care. "Father-offspring" and "Mother-offspring" represent the h^2 from father-offspring and mother-offspring regressions, respectively. "Parent-offspring" represents the h^2 value from both parents combined (i.e., midparent-midoffspring regression), and in parentheses is the value of combined h^2 corrected with an animal model. "Parental samples" describes the time when parents were sampled: as adults in an unspecified moment during their life ("adults"), as adults but during the specific breeding event ("breeding"), as nestlings, or not sampled. "Method" identifies the lab method used to measure telomeres: qPCR (quantitative real-time polymerase chain reaction) versus TRF (telomere restriction fragment analysis). NA identifies those cases for which no information is available.

[a]All birds sampled after 15 mo old in a range of 1–35 yr old.

[b]Only mother sampled; a statistically significant negative interaction between mother relative telomere length (RTL) and daughter RTL, and no relation with son RTL. h^2 was not estimated.

[c]All birds were sampled as breeding adults, both offspring and parents, but pedigree was reconstructed for several generations with breeding data.

[d]Positive correlation with foster mother.

[e]Sib-sib heritability, no parental sampling.

[f]h^2 was calculated for paternal half-sibling and maternal half-sibling, as well as among full siblings ($h^2 = 1.18$); no parental sampling.

also affected by parental age (Heidinger and Young 2020). The effects of parental age on offspring RTL can be both genetic effects and environmental (rearing) effects (Criscuolo et al. 2017). These effects seem to be linked to gamete maintenance and age-dependent quality of parental care (Heidinger and Young 2020; Monaghan et al. 2020). Gamete quality (i.e., resulting from combined traits like, e.g., gamete viability and DNA integrity) may indeed decline with age because gametes of old parents are more likely to have DNA mutations and shorter telomeres (Monaghan and Metcalfe 2019). Also, the quality of parental care (i.e., resulting from combined traits like, e.g., incubation, brooding, offspring provisioning, and nest defense; hereafter, "parental investment") may decline with age, for example, if older parents are less able to acquire resources for their offspring or if partners mated with older individuals allocate less care (Lemaître and Gaillard 2017). However, positive associations with parental age may also arise if experienced breeders invest more in breeding (Dupont et al. 2018) and/or if more experienced breeders can hold superior territories and mates (Asghar et al. 2015), thus benefiting overall offspring condition.

Parental phenotypic traits that are strongly linked to fitness and life history traits are thus expected to affect offspring phenotype and longevity. A distinctive type of phenotypic polymorphism, melanin-based color polymorphism, has been linked with variation in parental effort (Emaresi et al. 2014; Sumasgutner et al. 2016; Tate et al. 2016; Morosinotto et al. 2020; Nebel et al. 2020). This is because color morphs are expected to be adapted to different environmental conditions (Roulin 2004), with morph-specific physiological and behavioral profiles (Krüger 2002; Brommer et al. 2005; Ducrest et al. 2008; Linnen and Hoekstra 2009; Hubbard et al. 2010; Karell et al. 2011; Roulin and Ducrest 2011; Morosinotto et al. 2020), which may affect parental investment. Melanin-based color polymorphism has also been shown to be tightly linked to telomere dynamics in tawny owls (*Strix aluco*; Karell et al. 2017) where adults of the brown morph, which provide higher parental effort (Emaresi et al. 2014), have faster telomere shortening in erythrocytes than gray adults. Previous studies suggested that in birds there is a strong positive correlation in RTL in erythrocytes and other tissues (Reichert et al. 2013), including sperm RTL (although telomeres are generally longer in sperm; e.g., Delany et al. 2000; Kucera 2018). Thus, if offspring RTL depends mostly on the telomeres in parental gametes, we could expect that offspring of brown parents will have shorter telomeres, since their parents have a faster telomere-shortening rate. However, the existence of intergenerational effects of parental color on offspring RTL is currently still unclear, since there were no morph-specific differences in RTL among offspring (Morosinotto et al. 2021), and color of the offspring strongly depends on that of parents (Karell et al. 2011; Morosinotto et al. 2020). The current scenario is further complicated by a possible parental age effect. Indeed, in this species, morpho-specific telomere dynamics in adults are evident among experienced breeders (Karell et al. 2017) but do not appear among inexperienced breeders (i.e., adults at their first breeding attempt; Morosinotto et al. 2021). Therefore, we can expect that intergenerational effects of parental morphs would appear in this species only when parents get older, in sort of a color polymorphism–specific Lansing effect.

Here, we investigated first whether RTL is heritable in this species, as previously observed in other avian species (table 1), and second the impact of parental traits (age and color morphs of both parents) on the RTL of the offspring at fledging. We expect offspring RTL to be negatively affected by parental age owing to a decrement in parental investment, as previously observed in other species (table 2). Third, we tested the combined effects of color morph by age, since we expect that if offspring of brown parents inherit shorter telomeres, this effect should be particularly marked in offspring of older brown parents (i.e., morph × age interaction).

Methods

Blood samples were collected between 2013 and 2019 from a well-established tawny owl population with approximately 200 nest boxes spread in ca. 500 km^2 in western Uusimaa, southern Finland (60°15′N, 24°15′E). In early spring, all of the nest boxes were checked to detect breeding attempts and to collect information on clutch size, brood size, and hatching date. Parents were trapped in each nest a few days after hatching; they were aged, measured, and ringed to allow individual identification (Karell et al. 2009). Parents were aged as 1 yr old, 2 yr old, and 3 yr old or older (Karell et al. 2009) according to their partial molt (Karell et al. 2013). Plumage color was scored from facial disc, breast, back, and overall appearance to obtain a continuous color score that exhibited a clear bimodal pattern and was thus categorized as either gray morph or brown morph (Brommer et al. 2005). A blood sample was collected from the brachial vein of each parent at each breeding event, just after offspring hatching, and stored at −20°C until DNA extraction (except in 2013, when it was stored at −80°C; see below). At approximately 25 d old (age estimated from wing length), all of the offspring were weighed and color scored (Morosinotto et al. 2020). A blood sample was also collected from the brachial vein and stored at −20°C until DNA extraction and molecular sexing (except in 2013; see below).

Laboratory Analyses

Blood samples were collected as red blood cells from both parents and offspring directly in −80°C (2013), or they were stored in ethanol (2014, 2016–2018) or SET buffer (2019) and then kept at −20°C. DNA was extracted from all of the samples in 2019 using ammonium acetate (NH_4Ac) following a modification of the protocol by Nicholls et al. (2000); see details on the extraction method in Morosinotto et al. (2021). After DNA extraction, all of the offspring samples were sexed using a polymerase chain reaction (PCR)–based method to measure sex-specific narrow-sense heritability (h^2) using a modified protocol from Kekkonen et al. (2008) and Morosinotto et al. (2021).

Relative Telomere Length. The quantitative real-time PCR (qPCR) protocol for RTL estimation was modified from Karell et al.

Table 2: Summary of published literature on parental age effects on offspring relative telomere length (RTL) in avian species

Source	Species	Exp	Sex roles	Parental age effect	Gamete vs. parental care	Method
	Positive association: the older the parents, the longer the offspring RTL					
Asghar et al. 2015	Great reed warbler	No	B	Mother	Parental care	qPCR
Dupont et al. 2018	Black-browed albatross	No	B	Parental	Parental care	Southern blot
Sparks et al. 2021	Seychelles warbler	No	B	Mother	Both possible	qPCR
Brown et al. 2021	White-browed sparrow weaver	No	B	Maternal and paternal	Both possible	qPCR
	Negative association: the older the parents, the shorter the offspring RTL					
Heidinger et al. 2016	European shag	No	B	Parental	Parental care	qPCR
Criscuolo et al. 2017	Alpine swift	Yes	B	(Genetic) father (Foster) mother	Father: gamete Foster mother: parental care	qPCR
Bouwhuis et al. 2018	Common tern	No	B	Father	Gamete	TRF
Noguera et al. 2018	Zebra finch	Yes[a]	B	Father	Gamete	qPCR
Bauch et al. 2019	Jackdaws	Yes	B	Father	Gamete	TRF
Marasco et al. 2019	Zebra finch	Yes[b]	B	Mother	Both possible	qPCR
Sparks et al. 2021	Seychelles warbler	No	B	Father	Both possible	qPCR
This study	Tawny owl	No	C	Father	Parental care	qPCR
	No associations with parental age					
Öst et al. 2020	Eider duck	No[c]	A	No mother effects	NA	qPCR
Belmaker et al. 2019	Tree swallow	Yes	B	No parental effects	NA	TRF

Note. Experimental manipulation (exp; i.e., cross-fostering or experimental mating according to age) is marked as yes/no. "Sex roles" refers to sex roles in parental care classified as A for only female care, B for both parents providing equal parental care, and C for distinct sex roles in parental care. "Parental age effect" summarizes whether it is maternal, paternal, or both parents indistinctly. "Gamete vs. parental care" distinguishes between a parental age effect driven via gamete quality or age-mediated parental care quality (classified according to what was stated or suggested as most probable in each paper). "Method" identifies the lab method used to measure telomeres: qPCR (quantitative real-time polymerase chain reaction), TRF (telomere restriction fragment analysis), or Southern blot.

[a]Mother age experimentally controlled.

[b]Father age experimentally controlled.

[c]Father not tested.

(2017) and Morosinotto et al. (2021). For each sample, we ran two different real-time PCR plates: one with primers for an ultraconserved single-copy nuclear noncoding sequence (*sfsr*/3Fb 5′-ACTAGCCCTTTCAGCGTCATGT-3′ and *sfsr*/3Rb 5′-CATGCTCGGGAACCAAAGG-3′; Asghar et al. 2011; Karell et al. 2017) and one with primers for telomeres (*Tel*1b 50-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGT-T-3′ and *Tel*2b 5′-GGCTTGCCTTACCCTTACCCTTACCC TTACCCTTACCCT-3′; Criscuolo et al. 2009; Karell et al. 2017). Samples were incubated at 50°C for 2 min and at 95°C for 10 min, followed by 40 thermal cycles for *sfsr* primer (95°C for 15 s, 58°C for 45 s, and 72°C for 45 s) and 30 cycles for *Tel* primers (95°C for 15 s, 56°C for 30 s, and 72°C for 30 s). Both thermal protocols ended with a melt curve from 95°C reducing 0.5°C min^{-1} to exclude the presence of primer dimers. The qPCR used was a C1000 Touch termal cycler with a CFX96 real-time system (Bio-Rad). Each well of the qPCR plate contained 25 μL composed of 5 μL of DNA (2 ng μL^{-1}), 12.5 μL of Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen), 0.1 μL of ROX, the primers (0.3 μL at 10 μM for each *Tel* primer and 1 μL at 10 μM for each *sfsr* primer), and double-distilled H_2O (ddH_2O). Each 96-well plate included samples, two negative controls, and serially diluted standards (i.e., a randomly chosen tawny owl sample diluted two times with ddH_2O from 8 to 0.25 ng μL^{-1}). All plates also included an interplate control (i.e., a reference sample; 2 ng μL^{-1}) to control for interplate variability. All samples, negative and interplate controls, and standards were loaded in duplicates; samples were placed in pairs randomly in the plates, whereas the standards were loaded in the same well positions. Cycle threshold (Ct) values of intraplate sample duplicates had high repeatability for both primers (Ct for *sfsr*, $R \pm$ SE: 0.99 $\pm$ 0.001, confidence interval: 0.989–0.993, $P \leq 0.001$; Ct for *Tel*, $R \pm$ SE: 0.98 $\pm$ 0.002, confidence interval: 0.975–0.985, $P \leq 0.001$) and were measured using the rptr R package (Stoffel et al. 2017). The qPCR plates were discarded and rerun if the standard curves were outside the 100% $\pm$ 15% qPCR efficiency range (mean efficiency $\pm$ SD: *sfsr*, 94.5 $\pm$ 4.8; *Tel*, 92.4 $\pm$ 2.6). Efficiency was calculated on the regression of all data points (whole-curve fit) rather than on a single threshold, allowing the efficiency calculation to be only in points with good fluorescence reading (Zhao and Fernald 2005).

From all of the samples, we calculated a RTL (Criscuolo et al. 2009) adjusted for intra- and interplate assay variability using the

interplate control (Cawthon 2002; Karell et al. 2017; Morosinotto et al. 2021). The coefficient of variation of the RTL of the interplate control sample, corrected by the standard at dilution of 2 ng μL^{-1}, was 6.9% (RTL mean ± SD: 1.01 ± 0.07). RTL was calculated for all of the samples and was then standardized (mean: 0, SD: 1) to improve comparability with other studies, as suggested by Verhulst (2020).

Statistical Methods

Overall, we measured RTL in 290 samples: 178 fledglings and 112 samples from breeding adults. From this full data set, we calculated h^2 by multiplying the slope of the mother-midoffspring and father-midoffspring linear regression ($h^2 = b \times 2$). Of the 112 adult samples, 59 were maternal samples from overall 42 breeding individuals, and 53 were paternal samples from 39 individuals. We estimated h^2 for all broods according to maternal and paternal RTL measured during each breeding event (i.e., individual parents that were present in multiple years in the data set had a RTL estimated for each breeding event, only one breeding event occurred per year). In addition, we measured h^2 from mother-middaughter and mother-midson regressions and from father-middaughter and father-midson regressions. Moreover, h^2 was a posteriori calculated to see the effect of father age on the estimate of h^2 and the effect of year from both parents (see table A1). Our study system meets the assumptions of parent-offspring regressions, since no inbreeding and assortative mating are observed in this system. RTLs of the parents within a pair are only weakly correlated (Pearson's $r = 0.31$, $t = 2.27$, df = 48, $P = 0.03$), further supporting the lack of assortative mating in this system. There is also no genotype × environment interaction that could affect the h^2 estimates, since all individuals, both parents and offspring in each brood, were sampled within each breeding attempt, and all samples came from the same population, thus sharing the same environmental conditions. The h^2 values might, however, be partly underestimated owing to the fact that parents and offspring were sampled at different ages; however, since this was done in the same way for the entire data set, the biological patterns observed between the parents and offspring heritability should be maintained.

We also used an animal model approach to estimate heritability of RTL in the tawny owls. The animal model is a linear mixed model (LMM) that uses the relatedness between individuals to estimate the additive genetic variance (Kruuk 2004). We used the software ASReml-R version 4.1.0.143 (https://www.vsni.co.uk). We fitted individual ID (to take into account repeated measures and estimate nongenetic permanent environmental variance), the pedigree (to estimate additive genetic variance), and year (to estimate between year environmental variance) as random effects. In addition, we investigated the contribution of environmental parental and brood effects by fitting mother ID (to estimate maternal effects), father ID (to estimate paternal effects), and brood ID (to estimate brood effect) in separate models. Statistical significance of the random factors was determined with model comparisons using the likelihood ratio test with 1 df (Pinheiro and Bates 2000; Wilson et al. 2010). Furthermore, we tested the effect of father age on RTL as a fixed effect (see table B1).

After investigating heritability (with both parent-offspring regression and animal model), we ran a separate analysis to investigate whether offspring RTL is affected by parental phenotypic traits. To do so we ran a LMM on a smaller data set considering only data for complete families. The final sample size was thus 50 broods (7 in 2013, 8 in 2014, 11 in 2016, 6 in 2017, 5 in 2018, and 13 in 2019), with 147 offspring and 50 pairs of adults (for number of adults per morph per age class, see table A2). Offspring RTL according to parental traits was thus measured using a LMM with brood ID (i.e., a unique code identifying each nest) and year as random factors to take into account both nonindependence of offspring within the same brood and environmental variation, as well as storage method, across years. Some of the parents bred in multiple years: 10 females had multiple broods (each with two or three broods) among the overall 37 mothers, and 8 males had multiple brood (each with two to five broods) among the overall 36 males. However, for each brood, offspring RTL is compared exclusively with parental age and RTL measured at that specific breeding attempt. We nonetheless further included mother ID and father ID as random factors to control for the possible nonindependence of broods from the same parents. The covariates included in the model were color morph (gray or brown), age class (1 yr old, 2 yr old, 3 yr or older), and RTL of both parents, measured during each breeding event just after hatching of the brood. The father age × morph and mother age × morph interactions were included to test our predictions of the morph-specific impact of parental age (table 3). The interactions were removed from the models if not significant to detect the main effects of the variables included; the simplified model is presented in table A3. Storage method did not affect RTL measurements in this data set or in a larger data set measured at the same time (Morosinotto et al. 2021), and thus it was not included in the final model.

All the analyses were run in R version 3.6.1 (R Development Core Team 2019). Residuals were inspected visually to detect distribution patterns, and Grubbs test was performed on the data set to detect outliers (outliers package; Grubbs 1950). According to this test, one brood was an outlier in all of the data sets. We ran all of the analyses both with and without this outlier, but the results were qualitatively the same, and we thus retained the full data set, as it is representative of biological variation. The degrees of freedom were calculated with Satterthwaite's method, and the model was run with normal (Gaussian) error distribution. *P* values were calculated by likelihood ratio χ^2 tests (Anova function in car R package; Fox and Weisberg 2019). LMM was conducted with the lmer function within the lme4 package (Bates et al. 2015). Data have been deposited in the Dryad Digital Repository (https://doi.org/10.5061/dryad.6q573n619; Morosinotto et al. 2022).

Results

Offspring RTL at fledging was strongly positively associated with the RTL of both the father and the mother at the breeding event (table 3; fig. 1). The father-midoffspring linear regression revealed a

Table 3: Linear mixed model of offspring relative telomere length (RTL) according to parental standardized RTL, age (1 or 2 yr or older), and morph (gray or brown)

Variable	Slope ± SE	df	χ^2	P
Mother RTL	.40 ± .14*	1	7.81	**.005**
Mother age:				
2 yr	.01 ± .11	2	2.11	.35
Older	.07 ± .10	...	...	...
Mother morph (brown)	.11 ± .11	1	1.33	.25
Mother age × morph:				
2 yr × brown	−.07 ± .14	2	.34	.85
Older × brown	−.07 ± .13	...	...	...
Father RTL	.35 ± .13*	1	7.68	**.006**
Father age:				
2 yr	.06 ± .09	2	8.11	**.002**
Older	−.07 ± .08	...	...	...
Father morph (brown)	.11 ± .12	1	1.63	.20
Father age × morph:				
2 yr × brown	−.17 ± .13	2	1.76	.41
Older × brown	−.16 ± .13	...	...	...
	Variance ± SD			
Random effect:				
Brood ID	.003 ± .06			
Mother ID	.000 ± .000			
Father ID	.003 ± .06			
Year	.000 ± .000			
Residual	.016 ± .128			

Note. Offspring RTL: 2013–2019, $n = 147$, 50 broods, 37 mothers, and 36 fathers. For the class variables morph and age, gray and 1 yr old are used as the reference level, respectively, both for the main effects and for the interaction, and slope for each level is presented. P values for the whole variables are calculated with the Anova function (see "Methods"). Offspring and parental RTL were standardized to mean of zero and SD of one. Values in bold are statistically significant ($P < 0.05$). See table A3 for the main effect of father age in the simplified model without interactions (table A3; fig. A1).

*Significant according to the t-test in the summary of the model.

h^2 of 1.04 (linear regression: $b = 0.52 \pm 0.14$, $t = 3.78$, $P = 0.0004$; fig. 1*A*), with numerically different heritability estimated for daughters ($h^2 = 1.0$; linear regression: $b = 0.51 \pm 0.15$, $t = 3.43$, $P = 0.001$) and sons ($h^2 = 0.78$; linear regression: $b = 0.39 \pm 0.13$, $t = 3.09$, $P = 0.004$). The mother-midoffspring regression revealed a h^2 of 0.82 (linear regression: $b = 0.41 \pm 0.12$, $t = 3.49$, $P = 0.001$; fig. 1*B*), again with numerically different estimates for daughters ($h^2 = 0.9$; linear regression: $b = 0.45 \pm 0.12$, $t = 3.95$, $P = 0.0003$) and sons ($h^2 = 0.5$; linear regression: $b = 0.25 \pm 0.11$, $t = 2.34$, $P = 0.02$). The overall midparent-midoffspring regression revealed a h^2 of 0.48 (linear regression: $b = 0.48 \pm 0.10$, $t = 4.94$, $P = 0.001$; table 1). The animal models indicate that there is a significant additive genetic effect in RTL, with a heritability of 0.32 ± 0.09 (SE) in the best-fitted model (m3; see table B1 for the detailed animal models), which also takes into account year and permanent environmental effects. Estimates of maternal and paternal effects were very small and not statistically significant, and the models were not able to make proper estimates, since these effects were fixed at a boundary close to zero (table B1). There was, however, a small brood effect, although not statistically significant (table B1).

The RTL of offspring was also significantly affected by the age of the father at the breeding event (table 3) but not by the age of the mother (table 3). Offspring of older fathers (3 yr old or older) had significantly shorter telomeres than offspring of young fathers (1 and 2 yr old; table 3; see also table A3, fig. A1). The interactions of both maternal and paternal color morphs with parental age were not statistically significant (table 3; fig. 2), and parental color morph per se did not affect offspring RTL (table 3).

Discussion

Our results showed that RTL is highly heritable in tawny owls and seems to be inherited from both parents; daughters also show numerically slightly higher h^2 values toward both parents than sons. At the same time, offspring RTL was strongly dependent on the father's age, with offspring of older fathers having shorter telomeres, but there was no similar effect for the mother's age. Offspring RTL was not associated with the color morph of either parent, even though in tawny owls color polymorphism is a genetically determined trait (Karell et al. 2011; Morosinotto et al. 2020) that is known to be linked to survival (Karell et al. 2011) and life history traits (Brommer et al. 2005). We predicted shorter telomeres in offspring sired by brown parents, which show faster telomere shortening during adulthood than their gray conspecifics (Karell et al. 2017), assuming a positive correlation between RTL in erythrocytes and gametes (as suggested by Delany et al. 2000; Kucera 2018) owing to fast telomere loss in highly proliferative tissues (such as bone marrow and gonads; Haussmann et al. 2007). We predicted an association of parental color morph on offspring RTL, which could arise as a combined genetic and environmental effect. The genetic effect would be due to high heritability of both color and telomeres, whereas the environmental effect would be due to parental effort variation between morphs. In particular, we expected that this environmental effect would be stronger in older brown parents because of their higher reproductive investment and morph-specific senescence, but there was no evidence of such effects.

The strong correlation between offspring RTL and parental RTL at breeding suggests that RTL has a substantial heritability in this species, with a similar contribution from both parents. The animal models further indicate a significant additive genetic effect in RTL, while taking into account both year and permanent environmental effects. We were not able to fully estimate maternal and paternal effects in the animal models. This might be because the environmental maternal and paternal effects are indeed small but maybe more likely because there is a small proportion (around 30%) of parents that have bred more than once, and therefore these effects cannot be properly estimated. On the other hand, we were able to estimate a small effect of brood, which combined the effects of maternal, paternal, and sibling environmental

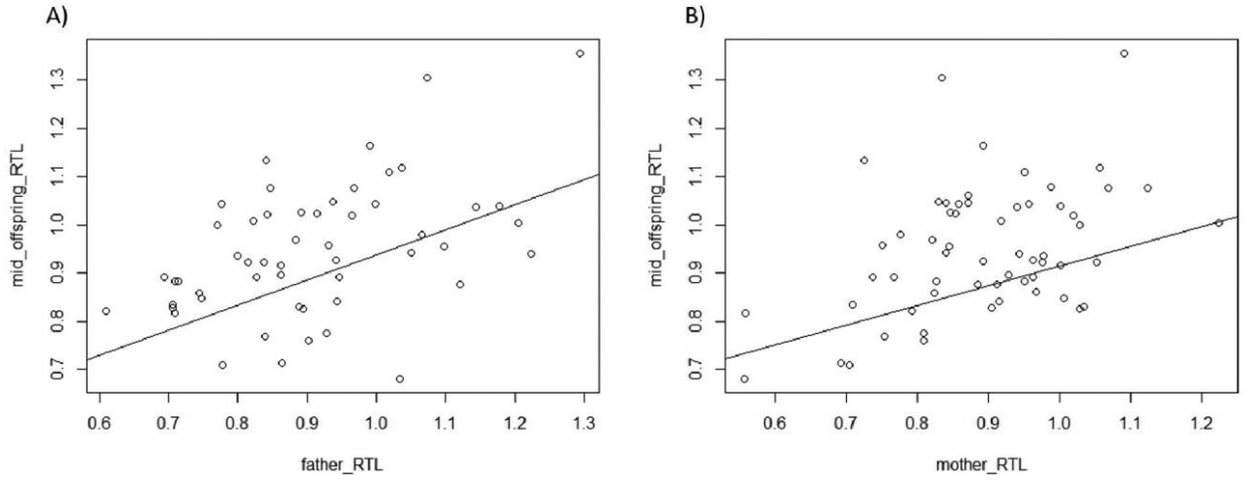

Figure 1. Linear regressions for father relative telomere length (RTL) and midoffspring RTL (average of brood RTL; heritability [h^2] = 1.04; *A*) and mother RTL and midoffspring RTL (h^2 = 0.82; *B*).

effects, although it was not statistically significant. In conclusion, we can say that RTL has a genetic component, but we could not detect any parental or brood effects using the animal model approach.

Our summary of avian studies on telomere heritability showed a large array of low-to-high and different heritability patterns (see table 1), going from maternally inherited (Asghar et al. 2015; Reichert et al. 2015) to high heritability from both parents (Bauch et al. 2019; Belmaker et al. 2019; this study). One possible explanation for the different heritability patterns observed may be found in the different sexual roles parents take in reproduction, through environmentally mediated effects (Dugdale and Richardson 2018). In tawny owls, we observe strong associations from both parents to their offspring. According to parent-offspring regression this association appeared numerically slightly stronger from fathers, who are the main food providers for the nestlings, while it was slightly weaker from mothers, who mainly defend the nest and brood the young. However, parent-offspring estimates of h^2 are imprecise and might be biased, making it challenging to evaluate whether the h^2 between parents is indeed significantly different. In the currently published literature, different heritability patterns do not seem to be consistently linked with sex roles in reproduction (see table 1). For example, maternal heritability is observed both in species with mostly or higher parental effort from females (Horn et al. 2011; Asghar et al. 2015; Öst et al. 2020) and in species with equal effort in parental care between parents (Becker et al. 2015; Reichert et al. 2015). This is only a qualitative comparison though and based on the few available studies. We thus conclude that, at the moment, there are not enough studies to strongly link heritability patterns with the role parents have during parental care. Moreover, despite the large variation in h^2 estimates between studies, very little can be said about phylogenetic variation in inheritance patterns because of both the possible strong variations across populations within species and the few phylogenetic groups tested. So far, telomere heritability has indeed been tested mostly in Passeriformes (zebra finch, *Taeniopygia guttata*; dipper, *Cinclus cinclus*; tree swallow, *Tachycineta bicolor*; collared flycatcher, *Ficedula albicollis*; great reed warbler, *Acrocephalus arundinaceus*; Seychelles warbler, *Acrocephalus sechellensis*; jackdaws, *Coloeus monedula*), with few studies on Sphenisciformes (king penguin, *Aptenodytes patagonicus*), Psittaciformes (kakapo, *Strigops habroptilus*), Anseriformes (eider, *Somateria mollissima*), and Strigiformes (tawny owl; this study; see table 1). More studies assessing h^2 of telomeres are needed to understand the diversity of associations between parent and offspring RTL in wild populations within and between species.

Offspring telomeres were overall shorter when the father was 3 yr old or older, whereas there was no link with maternal age, as previously observed in a few other bird species (see summary of recent studies in table 2). This result could be due to gametes of older males being of lower quality (Monaghan and Metcalfe 2019). Alternatively, older males might not be able to provide as high parental investment as younger males (Lemaître and Gaillard 2017), leading to a cost for the offspring (reduced resources) during their nestling growth. Tawny owls are long-lived birds, with rare records of individuals living up to 19 yr (P. Karell, unpublished data). Females stay mostly at the nest during incubation and the early nestling phase to brood the young and protect the nest from predators, while males hunt for the whole family. Thus, if older males are less efficient in hunting, this would result in reduced food provisioning to the offspring and may lead to lower body condition and shorter telomeres for the offspring. We can thus expect that a reduction in parental investment provided by older individuals would be especially visible for offspring of older fathers rather than mothers, although provisioning rate according to exact age has not yet been studied in this species. From a cross-fostering experiment on alpine swift (*Apus apus*), we know that indeed offspring RTL can also be negatively associated with the

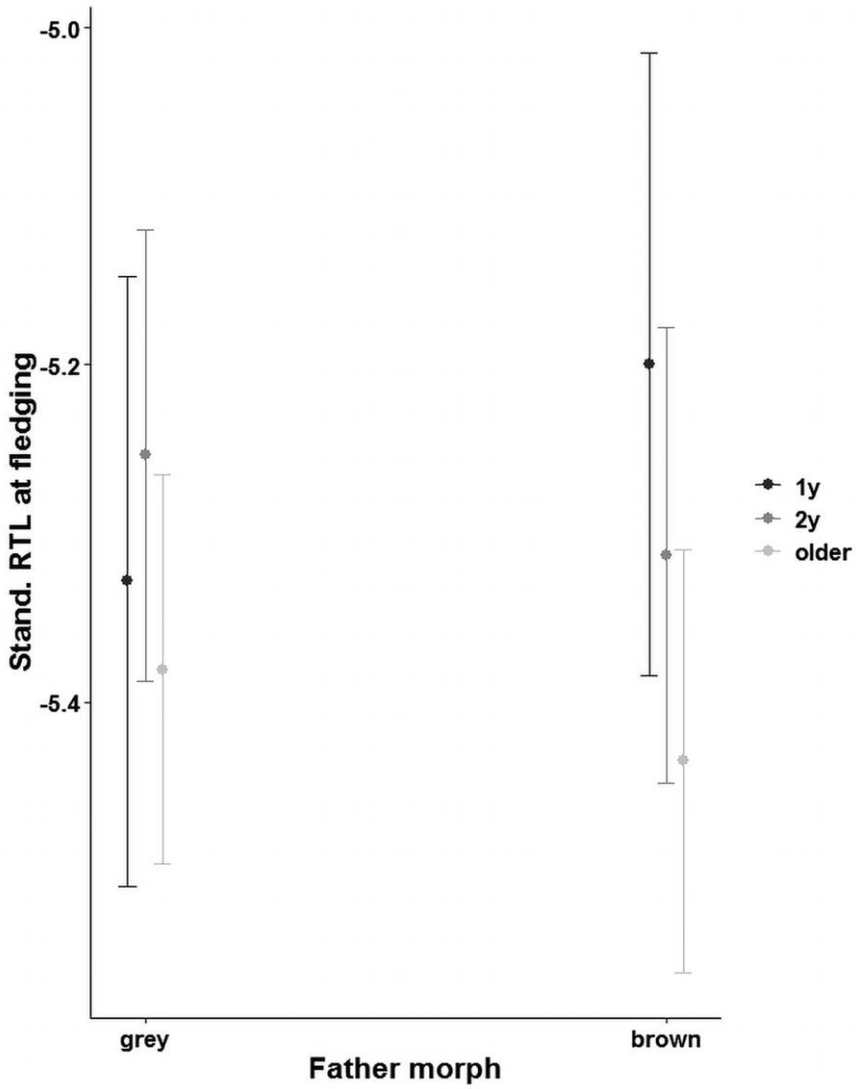

Figure 2. Estimates ± 95% confidence intervals of the linear mixed model on standardized offspring relative telomere length (RTL) at fledging according to the interaction between father age (1 or 2 yr old or older) and father morph (gray or brown; estimates from the interaction in table 3; for the main effect of father age, see fig. A1).

age of foster parents, probably via reduced parental investment (Criscuolo et al. 2017). In our data set all of the offspring were sampled just before fledging (approximately at day 25), and a cross-fostering experiment was not feasible owing to logistic constraints, and we thus cannot distinguish which mechanism (gamete vs. parental care effects) is at play.

Offspring RTL was not affected by the interactions of parental age and color morphs. We predicted that offspring of older brown parents would have shorter telomeres than offspring of older gray parents because brown adults show shorter telomeres and faster telomere shortening during their breeding lifetime (Karell et al. 2017). Since this difference is evident among adult experienced breeders (Karell et al. 2017) but not among inexperienced breeders (Morosinotto et al. 2021), we expected that an effect of parental color morph would be more evident in older parents. Assuming that the genetic versus environmental effects in our estimates are constant and that RTL in erythrocytes is positively correlated with that in gametes (as suggested by Kucera 2018), we expected that if offspring telomeres are affected mostly by parental telomeres in the germ line, offspring of older brown parents would inherit short telomeres. The lack of an interactive morph-specific effect by age thus suggests either that shorter erythrocyte telomeres in older brown adults do not translate to substantially shorter telomere in gametes or that there is some compensatory effect that overcomes the "negative start" for offspring of brown parents during the nestling growth period. A possible explanation of the pattern observed is that brown adults might be able to provide

higher parental investment and resources (Emaresi et al. 2014) and can thus compensate for possible inherited costs. In this scenario, offspring of brown parents might catch up during growth and ultimately be in similar or even better condition at fledgling (i.e., when sampled) compared with offspring of gray adults. Indeed, previous results showed that brown offspring (and offspring of brown pairs) have consistently higher mass at fledgling independently of food abundance (Morosinotto et al. 2020) and that offspring of brown mothers gain more weight than offspring of gray mothers under controlled ad lib. food conditions (Piault et al. 2009).

The current results combined overall show that in this species, telomeres are highly heritable and that paternal age has a negative impact on offspring RTL, probably through reduced foraging efficiency in older males but that this Lansing effect is not color morph specific. Thus, the previously observed morph-specific patterns in telomere dynamics emerge only as a consequence of different life history strategies adopted in adulthood (Karell et al. 2017) and do not seem to affect offspring RTL (this study; Morosinotto et al. 2021). Previous studies in other species found different telomere dynamics according to adult color polymorphism linked to morph-specific life history strategies, for example, in the Australian painted dragon (*Ctenophorus pictus*; Rollings et al. 2017), and according to offspring melanin coloration, for example, in swallows (*Hirundo rustica*; Costanzo et al. 2017). However, as far as we know, this is the first study investigating whether morph-specific telomere dynamics in aging adults explains offspring RTL and the first study exploring whether sex roles in parental care play a role in shaping parent-offspring associations in RTL. Further studies involving experimental manipulation to separate genetic and environmental effects are needed to identify whether the lack of a morph-specific aging effect and the negative effect of paternal age are linked to parental investment or gamete quality. Further investigations are also needed to determine how inheritance of telomere dynamics vary across species and the reason behind the various patterns observed.

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APPENDIX A

Table A1: Estimates of heritability (h^2) with age and year in the model to control for age effect and year/storage effects, respectively

	Parent-offspring regression with year	Parent-offspring regression with father age
Mother-midoffspring	.80	...
Mother-sons	.46	...
Mother-daughters	.92	...
Father-midoffspring	1.05	1.08
Father-sons	.82	.84
Father-daughters	.99	1.05

Note. See text for overall h^2 estimates from both parent-offspring regressions and the animal model. Only father age was considered, since according to tables 3 and A3, no effect of mother age on offspring telomere length was detected. For the animal models (including year and age effects), see table B1.

Table A2: Sample sizes of the parents per age and color

Age (yr)	Gray females	Brown females	Gray males	Brown males
1	2 (6)	4 (6)	3 (5)	2 (5)
2	10 (16)	6 (16)	8 (13)	5 (13)
3 or older	18 (28)	10 (28)	19 (32)	13 (32)

Note. $N = 50$ broods in 6 yr (30 gray and 20 brown individuals in both sexes). The total number of individuals per sex in each age class is given in parentheses.

Table A3: Simplified linear mixed model of offspring relative telomere length (RTL) according to parental standardized RTL, age (1 or 2 yr old or older), and morph (gray or brown)

Variable	Slope ± SE	df	χ^2	P
Mother RTL	.40 ± .13*	1	8.74	**.003**
Mother age:				
2 yr	−.02 ± .06	2	1.51	.47
Older	.03 ± .06	...	...	...
Mother morph (brown)	.03 ± .04	1	.74	.39
Father RTL	.32 ± .12*	1	7.05	**.008**
Father age:				
2 yr	−.01 ± .06	2	9.67	**.008**
Older	−.13 ± .06*	...	...	...
Father morph (brown)	−.04 ± .04	1	1.09	.30
	Variance ± SD			
Random effect:				
Brood ID	.003 ± .05			
Mother ID	.000 ± .000			
Father ID	.004 ± .06			
Year	.000 ± .000			
Residual	.02 ± .13			

Note. Offspring RTL: 2013–2019, 50 broods, $n = 147$. For the class variables morph and age, gray and 1 yr old are used as the reference level, respectively, both for the main effects and for the interaction, and slope for each level is presented. P values for the whole variables are calculated with the Anova function (see "Methods"). Offspring and parental RTLs were standardized to mean of zero and SD of one. Values in bold are statistically significant ($P < 0.05$). See table 3 for the full model with interaction.

*Significant according to the t-test of the model.

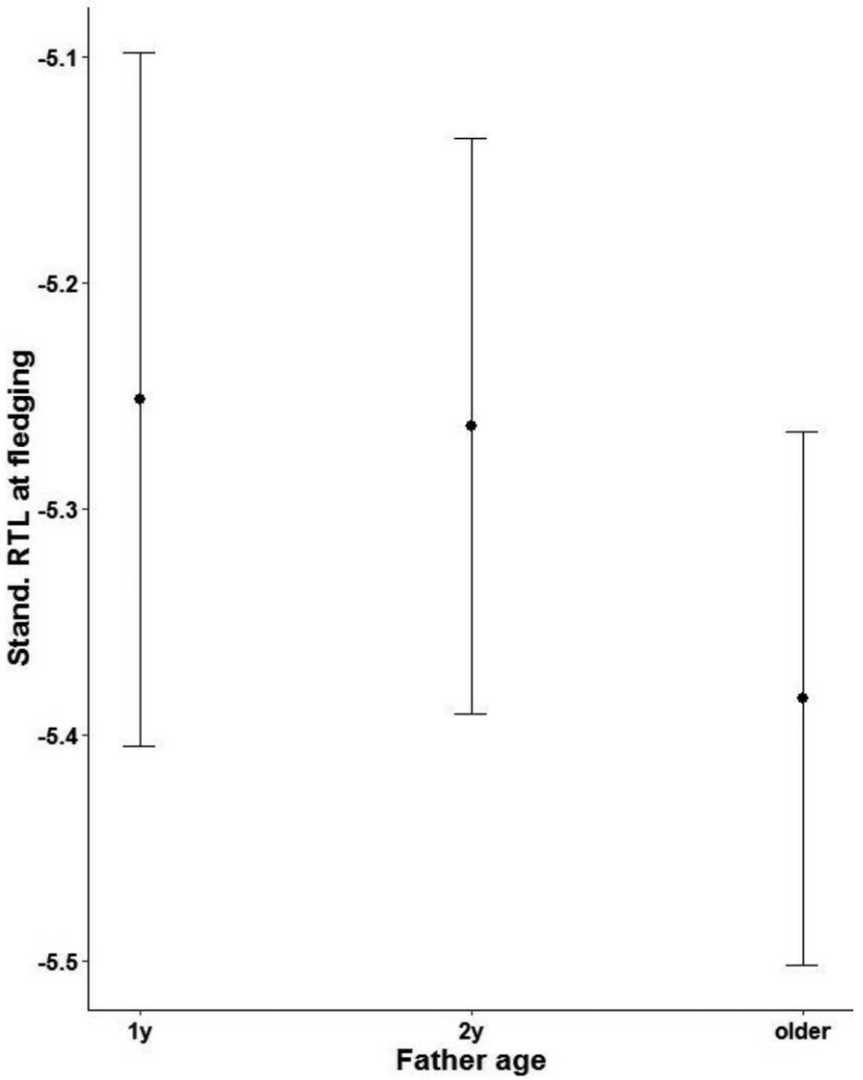

Figure A1. Estimates ± 95% confidence intervals of the linear mixed model on standardized offspring relative telomere length (RTL) at fledging according to father age (1 or 2 yr old or older); see table A3 for the main effect of father age and figure 2 for the graph of father age by morph.

APPENDIX B

Table B1: Estimated additive genetic and parental effects of tawny owl telomere length using an animal model approach

Model no.	V_{PE} ± SE	V_A ± SE	V_{YEAR} ± SE	V_M ± SE	V_F ± SE	V_{BROOD} ± SE	V_E ± SE	Perm env ± SE	h^2 ± SE	Year ± SE	Maternal ± SE	Paternal ± SE	Brood ± SE	df	LogLik	Model comparison (test variable: *P*)	Fixed effect *P*
m0	.011 ± .004						.015 ± .004	.426 ± .136						289	382.953		
m1	.000 ± .000	**.009 ± .003**[a]					.015 ± .002	.007 ± .010	**.375 ± .092**[a]					289	404.424	m1 vs. m0 (V_A: 5.63E–11)	
m2	.013 ± .003		.005 ± .004				.010 ± .002	.453 ± .111		.196 ± .110				289	404.140		
m3	.000 ± .000	**.008 ± .003**[a]	**.004 ± .003**[a]				.013 ± .002	.004 ± .006	**.324 ± .093**[a]	**.149 ± .094**[a]				289	416.109	m3 vs. m1 (year: 1.34E–06); m3 vs. m2 (V_A: 9.95E–07)	
m4	.000 ± NA	.017 ± .005	.004 ± .003	.000 ± NA[a]			.009 ± .003	.000 ± .000	.556 ± .145	.138 ± .095	.000 ± .000[a]			192	263.252	m4 vs. m3 (mother: 1)	
m5	.000 ± NA	.019 ± .006	.004 ± .003		.000 ± NA[a]		.008 ± .003	.000 ± .000	.600 ± .144	.140 ± .095		.000 ± .000[a]		187	256.622	m5 vs. m3 (father: 1)	
m6	.000 ± NA	.013 ± .006	.004 ± .003			.003 ± .003[a]	.010 ± .004	.000 ± .000	*.435 ± .189*	.122 ± .091			.089 ± .089[a]	193	265.677	m6 vs. m3 (brood: 1)	
m7	.018 ± .007						.011 ± .006	.612 ± .206						174	216.295		
m8	.000 ± NA	**.019 ± .006**[a]					.012 ± .004	.000 ± .000	**.621 ± .145**[a]					174	226.332	m8 vs. m7 (V_A: 7.45E–06)	.098
m9	.020 ± .004		.005 ± .004				.006 ± .003	.638 ± .141		.160 ± .102				174	224.834		
m10	.000 ± .000	**.019 ± .006**[a]	**.005 ± .004**[a]				.009 ± .004	.001 ± .002	**.574 ± .157**[a]	**.153 ± .104**[a]				174	233.082	m10 vs. m8 (year: .00024); m10 vs. m9 (V_A: .00026)	.194
m11	.000 ± .000	.016 ± .007	.004 ± .004				.009 ± .004	.001 ± .002	.496 ± .196	.140 ± .101			.065 ± .088[a]	174	233.509	m11 vs. m10 (brood: .355)	.258

Note. Models m0 through m6 include only population mean as a fixed effect, while models m7 through m11 include father age (factor with three categories) as a fixed effect. The best-fitted model is m3. The following models were fitted: m0: fixed = TS ~ 1, random = ~ ANIMAL; m1: fixed = TS ~ 1, random = ~ ANIMAL + GENETIC; m2: fixed = TS ~ 1, random = ~ ANIMAL + Year; m3: fixed = TS ~ 1, random = ~ ANIMAL + GENETIC + Year; m4: fixed = TS ~ 1, random = ~ ANIMAL + GENETIC + Year + Mother; m5: fixed = TS ~ 1, random = ~ ANIMAL + GENETIC + Year + Father; m6: fixed = TS ~ 1, random = ~ ANIMAL + GENETIC + Year + BroodID; m7: fixed = TS ~ 1 + Father age, random = ~ ANIMAL; m8: fixed = TS ~ 1 + Father age, random = ~ ANIMAL + GENETIC; m9: fixed = TS ~ 1 + Father age, random = ~ ANIMAL + Year; m10: fixed = TS ~ 1 + Father age, random = ~ ANIMAL + GENETIC + Year; m11: fixed = TS ~ 1 + Father age, random = ~ ANIMAL + GENETIC + Year + BroodID. TS = telomere length. The random factors in the models are as follows: ANIMAL = permanent environmental variance (V_{PE}); GENETIC = additive genetic variance (V_A); Year = year variance (V_{YEAR}); Mother = maternal variance (V_M); Father = paternal variance (V_F); BroodID = brood variance (V_{BROOD}). These are accompanied with their standard errors (SEs). The variances are also presented as proportions of the total phenotypic variance, where the columns for perm env (permanent environment), h^2 (heritability), year, maternal, paternal, and brood show these respective effects. Significance testing was performed by comparing models with likelihood ratio tests, and each tested variable is presented with a *P* value. Boldface type indicates $P < 0.05$, and italic type indicates $P < 0.1$. The log likelihood (LogLik) and degrees of freedom (df) are presented for each model. The fixed effect was tested with a Wald test, and *P* values are presented. Variances with SE values given as NA were fixed at a boundary close to zero.

[a]Tested in each model.

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