

1 **Early-life telomere length covaries with life-history traits**
2 **and scales with chromosome length in birds**

3 **Running title: Avian telomere length evolution**

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16 **ABSTRACT**

17 Telomeres, the short DNA sequences that protect chromosome ends, are an ancient molecular
18 structure, which is highly conserved across most eukaryotes. Species differ in their telomere
19 lengths, but the causes of this variation are not well understood. Here, we demonstrate that mean
20 early-life telomere length is an evolutionary labile trait across 58 bird species (representing 35
21 families in 12 orders) with the greatest trait diversity found among passerines. Among these
22 species, telomere length is significantly negatively associated with the fast-slow axis of life-
23 history variation, suggesting that telomere length may have evolved to mediate trade-offs
24 between physiological requirements underlying the diversity of pace-of-life strategies in birds.
25 Curiously, within some species, larger individual chromosome size predicts longer telomere
26 lengths on that chromosome, leading to the suggestion that telomere length also covaries with
27 chromosome length across species. We show that longer mean chromosome length or genome
28 size tends to be associated with longer mean early-life telomere length (measured across all
29 chromosomes) within a phylogenetic framework constituting up to 32 bird species. Combined,
30 our analyses generalize patterns previously found within a few species and provide potential
31 adaptive explanations for the 10-fold variation in telomere lengths observed among birds.

32

33 **INTRODUCTION**

34 Telomeres are an ancient molecular structure which is conserved across most eukaryotes
35 (Fulnecková et al., 2013; Meyne, Ratliff, & Moyzis, 1989). Telomeres might have emerged
36 when linear chromosomes evolved from a circular chromosome ancestor around 1.5 billion years
37 ago (Lee, Leek, & Levine, 2017). Their function is to protect linear chromosomes during
38 incomplete end-replication (Blackburn & Szostak, 1984) and oxidative stress (von Zglinicki,

39 2002), and in most tetrapods mean telomere length (TL) of somatic cells shortens through life
40 (e.g. Tricola et al., 2018). Within some species, short telomeres have been shown to correlate
41 with shorter lifespan (Heidinger et al., 2012), increased size (Ringsby et al., 2015), and oxidative
42 stress (Reichert & Stier, 2017). Across species, a faster rate of telomere loss has been linked to
43 shorter maximum species lifespan in several studies (Dantzer & Fletcher, 2015; Haussmann et
44 al., 2003; Pepke & Eisenberg, 2020; Tricola et al., 2018; Vleck, Haussmann, & Vleck, 2003;
45 Whittemore, Vera, Martínez-Nevado, Sanpera, & Blasco, 2019). However, no consistent patterns
46 have emerged in how absolute TL is associated with lifespan or body mass across species
47 (Gomes et al., 2011; Haussmann et al., 2003; Pepke & Eisenberg, 2021; Seluanov et al., 2007;
48 Tricola et al., 2018).

49 The mean TL, measured across all chromosomes, has emerged as biologically relevant
50 trait in evolutionary, ecological, and physiological studies (Monaghan, 2010; Nussey et al.,
51 2014). Among wild birds, mean adult TL varies from around 5 kb in Western jackdaws (*Coloews*
52 *monedula*, Salomons et al., 2009) to more than 50 kb in great tits (*Parus major*, Tricola et al.,
53 2018), but an evolutionary explanation for this 10-fold difference in mean TL is lacking (Tricola
54 et al., 2018). A similar magnitude of variation is found within mammals (Gomes et al., 2011), in
55 which TL is shorter in larger and longer-lived species (Pepke & Eisenberg, 2021). Furthermore,
56 TL is positively associated with cancer risk across mammalian species (Pepke & Eisenberg,
57 2021). Thus, in mammals, evolution of shorter telomeres can be explained as an adaptation to
58 counteract the increased risk of development of tumors associated with a larger number of cells
59 and longer time to accumulate oncogenic mutations in larger and longer-lived species (Gomes et
60 al., 2011; Gorbunova, Seluanov, Zhang, Gladyshev, & Vijg, 2014; Pepke & Eisenberg, 2021;
61 Risques & Promislow, 2018; Seluanov et al., 2007; Tian et al., 2018). However, no association

62 between TL and lifespan has been found in birds (Hausmann et al., 2003; Tricola et al., 2018;
63 Vleck et al., 2003) and it is not known how TL covaries with body mass or other life-history
64 traits in birds.

65 In life-history theory, evolutionary trade-offs are expected between vital life-history
66 traits, such as between investment in current reproduction and investment in somatic growth,
67 maintenance, or future reproduction (Stearns, 1989). Organisms can be placed along a fast-slow
68 continuum of life-histories depending on how they resolve such trade-offs (Roff, 1993). A fast
69 pace-of-life is characterized by higher investment in reproduction over survival, which is
70 reflected in species with e.g. large clutch sizes and short generation times and lifespan (Araya-
71 Ajoy et al., 2021; Ricklefs & Wikelski, 2002; Sæther, 1988). The variation in pace-of-life
72 strategies is associated with physiological differences between species (Dammhahn,
73 Dingemanse, Niemelä, & Réale, 2018; Ricklefs & Wikelski, 2002). We hypothesize that TL may
74 have coevolved with suites of life-history traits and that TL may be used to rank species on the
75 slow-fast axis of variation in life-history traits. Short telomeres could reflect decreased
76 allocation of investment in somatic maintenance and hence increased allocation of resources to
77 reproduction (Giraudeau, Angelier, & Sepp, 2019; Monaghan, 2010). However, long telomeres
78 may be an adaptation to the cumulative negative effects of reproduction (Sudyka, 2019) and
79 rapid growth on TL (Pepke et al., 2021), revealing the opposite relationship between TL and
80 pace-of-life across species. We therefore investigate associations between TL and the slow-fast
81 axis of life-history variation across species (Dantzer & Fletcher, 2015).

82 Curiously, within several species, cytogenetic studies have shown a positive correlation
83 between the TL at a particular chromosome arm and the corresponding total chromosome length
84 or chromosome arm length (reviewed in Klegarth & Eisenberg, 2018). This pattern has been

85 found within laboratory mice (*Mus musculus*, Zijlmans et al., 1997), Chinese hamsters
86 (*Cricetulus griseus*, Slijepcevic & Hande, 1999), humans (*Homo sapiens*, Graakjaer et al., 2003;
87 Mayer et al., 2006; Suda et al., 2002; Wise et al., 2009), arabidopsis (*Arabidopsis thaliana*,
88 Shakirov & Shippen, 2004), pearl millets (*Pennisetum glaucum*, Sridevi, Uma,
89 Sivaramakrishnan, & Isola, 2002), yeast (*Saccharomyces cerevisiae*, Berthiau et al., 2006;
90 Craven & Petes, 1999), and Tetrahymena (*Tetrahymena thermophila*, Jacob, Stout, & Price,
91 2004). Klegarth and Eisenberg (2018) tested whether this relationship extends across mammal
92 species, using data on adult mean TL and mean chromosome length across 39 species of
93 Primates and Cetartiodactyla (Gomes et al., 2011) and 11 species of Rodentia (Seluanov et al.,
94 2007). They did not find any significant associations, but within Primates and Cetartiodactyla a
95 positive trend between TL and chromosome length became stronger when the outlier Indian
96 Muntjac (*Muntiacus muntjac*) was removed. Indian Muntjacs have recently undergone several
97 whole chromosome fusions resulting in very large chromosomes (Wang & Lan, 2000). Perhaps
98 an evolutionary lag accounts for their relatively short telomeres, which are adapted to shorter
99 chromosomes (Klegarth & Eisenberg, 2018). Furthermore, many of the species included in past
100 studies have been held in captivity or domesticated by humans, which might have altered their
101 telomere length dynamics (Eisenberg, 2011; Manning, Crossland, Dewey, & Van Zant, 2002;
102 Pepke & Eisenberg, 2021).

103 Birds may present an easier taxon with which to examine telomere–chromosome length
104 co-evolutionary dynamics: In contrast to mammals, the avian karyotype is relatively conserved,
105 and most birds have a chromosome number (2n) around 80 (Degrandi et al., 2020; Ellegren,
106 2010) suggesting that inter-chromosomal rearrangements are rare in birds. However, most bird
107 species possess several microchromosomes, which have been shown to harbor functional, but

108 ultra-long (“class III”) telomeres in some species (Atema, Mulder, van Noordwijk, & Verhulst,
109 2019; Delany, Gessaro, Rodrigue, & Daniels, 2007; Delany, Krupkin, & Miller, 2000; Nanda &
110 Schmid, 1994; Nanda et al., 2002; Rodrigue, May, Famula, & Delany, 2005). Not all
111 microchromosomes possess ultra-long telomeres (and not all species with microchromosomes
112 possess any ultra-long telomeres, Delany et al., 2000; Nanda & Schmid, 1994; Nanda et al.,
113 2002). Furthermore, these abnormal telomeres were found only on one chromosome arm and
114 only in some individuals of inbred domestic chickens (*Gallus gallus*), whereas TL of the
115 opposite chromosome arm was of typical size (“class II”, Delany et al., 2007). It is therefore
116 possible that potential telomere–chromosome dynamics vary across chromosome size ranges
117 (Atema et al., 2019), but this is unexplored in birds. Birds have the smallest genomes among
118 extant amniotes, which is generally thought to represent adaptations to the metabolic
119 requirements of active flight (Kapusta, Suh, & Feschotte, 2017; Wright, Gregory, & Witt, 2014).
120 Yet, compared to mammals, some birds seem to have a larger amount of telomere sequences
121 (Delany et al., 2000). Indeed, some of the ultra-long telomere signals may be attributed to sub-
122 telomeric repeats (“class P”, Ingles & Deakin, 2016, but see Atema et al., 2019), which occur in
123 some bird species (Nanda et al., 2002).

124 Here, we use mainly TL measurements of individuals of known age to obtain estimates of
125 an early-life TL. We first reconstruct the evolution of mean TL of 58 bird species. We then
126 investigate how TL relates to key life-history traits of birds. We then proceed to test the
127 hypothesis that TL covaries with chromosome length across species. We do not resolve within-
128 genome variation in TL across chromosomes, which is largely unknown in birds (Nanda et al.,
129 2002), but we test whether variation in mean chromosome length underlies some of the variation
130 observed in mean TL across species.

131

132 **MATERIALS AND METHODS**

133 **Telomere length data**

134 The use of methods to estimate relative amounts of telomeric DNA within samples
135 (qPCR) limits the feasibility of comparative studies (Nussey et al., 2014). In this study we only
136 used TL measured via the telomere restriction fragment (TRF) method (Hausmann & Vleck,
137 2002) or high-throughput quantitative fluorescence *in situ* hybridization (Q-FISH, Canela, Vera,
138 Klatt, & Blasco, 2007; Lansdorp et al., 1996). In the TRF analysis, mean TL value is obtained
139 from the distribution of TLs (in a Southern blot gel smear) across all chromosomes (Hausmann
140 & Mauck, 2008). In the Q-FISH analysis, mean TL is obtained from the mean telomere probe
141 fluorescence intensity across all chromosomes (quantified using microscopy image analysis,
142 Canela et al., 2007). We searched the literature for avian telomere studies using TRF to measure
143 mean TL from blood (Web of Science and Google Scholar [March 2021] search terms:
144 “telomere”, “TRF”, “restriction fragment”, “Q-FISH”, “avian”, or “bird”). Correlations between
145 TLs of various tissues suggest that blood TL is a good proxy of the TL across the organism
146 (Daniali et al., 2013; Demanelis et al., 2020; Reichert, Criscuolo, Verinaud, Zahn, & Massemin,
147 2013). We used TL estimates from the same lab when available to minimize methodological
148 effects (Tricola et al., 2018). Most studies used non-denatured in-gel TRF or Q-FISH to measure
149 functional (terminal) TLs, except for 15 species indicated in Table S1 in the Supporting
150 Information. These studies used denatured TRF, which may include short interstitial (class I)
151 telomeric sequences, that may lead to underestimation of the mean functional TL (Foote, Vleck,
152 & Vleck, 2013). The size distinction between class II and III telomeres is not well-defined
153 (Atema et al., 2019; Delany et al., 2000) and some studies may not include the complete TL

154 distribution if it is outside the range of the specific molecular size markers used (Atema et al.,
155 2019; Foote et al., 2013; Haussmann & Mauck, 2008). However, since chromosome-specific
156 TLs are largely unknown in birds, we rely on estimates of mean TL, which may reflect mainly
157 “class II” telomeres.

158 To estimate an early-life TL for species with TL measurements at different reported ages,
159 within each species we performed a linear regression of TL and individual age and used the
160 extrapolated TL value at age 0 (i.e. the intercept, see Fig. 1 in Tricola et al., 2018). For these
161 species, interspecific variance (97%) greatly exceeded intraspecific variance (3%) in TL,
162 suggesting that our age correction method will only slightly change interspecific TL
163 comparisons. For the remaining species, we used mean TL estimates for the youngest individuals
164 reported (all <1 year old), except for 8 species (Table S1), where TL is averaged across unknown
165 age classes. The domestic chicken was excluded because no mean TL has been reported (Delany,
166 Daniels, Swanberg, & Taylor, 2003; Delany et al., 2000) and the chicken has a long history of
167 human domestication, which is likely to have altered its telomere biology (Pepke & Eisenberg,
168 2021) and genome biology (Piégu et al., 2020). Variation in the activity of telomerase, a
169 ribonucleoprotein capable of rebuilding telomeres (reviewed in Criscuolo, Smith, Zahn,
170 Heidinger, & Haussmann, 2018), is unmeasured in our study and may confound the estimation of
171 early-life TL.

172 **Cytogenetic data**

173 For 20 species estimates of genome size (the amount of DNA in a haploid genome, i.e. c-
174 value) measured in erythrocytes were compiled from the Animal Genome Size Database, 2.0
175 (Gregory, 2020). When more than one c-value estimate was available in the database, we
176 followed Gregory (2020) and averaged c-values across studies using up to four comparable

177 cytometric methodologies (Hardie, Gregory, & Hebert, 2002, see Table S1). C-values (pg) were
178 converted to mega base pairs (Mb) by multiplying with 978 Mb/pg (Doležel, Bartoš, Voglmayr,
179 & Greilhuber, 2003). When these estimates were not available, we searched the National Center
180 for Biotechnology Information genetic sequence database GenBank (Clark, Karsch-Mizrachi,
181 Lipman, Ostell, & Sayers, 2016) and recorded the length of whole genome sequence (WGS)
182 assemblies (in Mb, 10 species, in addition to 2 species from Grayson, Sin, Sackton, & Edwards,
183 2017, Table S1). Genome size estimates from cytometric methods are highly correlated with data
184 obtained from WGS projects (Elliott & Gregory, 2015). Sequencing methods seem to
185 underestimate genome size in some cases, a discrepancy that increases with absolute genome
186 size (Elliott & Gregory, 2015). Since birds have relatively small genomes (1.0-2.2 Gb, Kapusta
187 et al., 2017), we do not try to correct for this, but use cytometric estimates when available.
188 Number of chromosomes was compiled from Degrandi et al. (2020). Atypical karyotypes are
189 known from birds (Damas, O'Connor, Griffin, & Larkin, 2019; de Boer & van Brink, 1982) and
190 we did not attempt to infer missing cytogenetic data from closely related species. Average
191 chromosome lengths were calculated by first dividing genome size by the number of (haploid)
192 chromosomes and then subtracting the telomeric DNA component from each chromosome
193 (average TL multiplied by 2 representing the number of telomere arms per chromosome). For 14
194 species the karyotype is not yet known, and we therefore also test the association between TL
195 and genome size, as a proxy for chromosome length across 32 species.

196 **Life-history data**

197 Data on maximum lifespan (years) and average adult body mass (g) were compiled
198 primarily from AnAge: The Animal Ageing and Longevity Database (Tacutu et al., 2018, Table
199 S1 and S2), which combines data from captive and wild populations. Mean clutch size (mean

200 number of eggs laid per clutch) and generation time (in years, based on modelled values of age at
201 first reproduction, maximum lifespan, and annual adult survival) were primarily compiled from
202 Bird et al. (2020), see Table S1. TL, mass, lifespan, and generation time were \log_{10} -transformed
203 to linearize relationships observed in bivariate plots. We then used a phylogenetic principal
204 component analysis (Revell, 2009) to construct a first principal component (PC1), which
205 explained 55% of the variation in these traits and may reflect the fast (low values) to slow (high
206 values) axis of variation in pace-of-life strategies (Table S3, Dantzer & Fletcher, 2015; Jeschke
207 & Kokko, 2009).

208 **Phylogenetic reconstruction**

209 We used the most recent time-calibrated avian phylogeny (Jetz, Thomas, Joy, Hartmann,
210 & Mooers, 2012) based on the Hackett et al. (2008) backbone. We compiled a set of 1,000 trees
211 from BirdTree.org and summarized these into a single maximum clade credibility tree using the
212 maxCladeCred function in the ‘phangorn’ package in R (Schliep, 2011). This tree was pruned
213 using the ‘ape’ package (Paradis & Schliep, 2018) and visualized using the ‘phytools’ package
214 (Revell, 2012). Ancestral states were estimated using the function ‘fastAnc’ (Revell, 2012).

215 **Phylogenetic comparative analyses**

216 Phylogenetic generalized least square regressions were performed using the ‘pgls’
217 function in ‘caper’ package (Orme et al., 2018), in which a variance-covariance matrix from the
218 phylogenetic relationships (branch lengths) is compared to the actual covariance structure in the
219 residual errors of the regression. The phylogenetic signal, λ , is a multiplier of the expected
220 covariances (off-diagonal elements) that produces the actual variance-covariance matrix
221 (Freckleton, Harvey, & Pagel, 2002; Pagel, 1999). Under the Pagel’s λ (PA) model, branch
222 length transformations are optimized numerically using maximum likelihood within default

223 bounds (0.0-1.0, Orme et al., 2018). When $\lambda=0$ the covariance between species is zero
224 corresponding to a non-phylogenetic (ordinary) regression, or “star model” (ST), normally
225 indicating that the traits are evolutionary very labile (Blomberg, Garland Jr, & Ives, 2003;
226 Kamilar & Cooper, 2013). When $\lambda=1$ the evolution of the residual error is best approximated by
227 a Brownian motion (BM) model of evolution (Felsenstein, 1985), which is the case for many
228 gradually evolving traits (Kamilar & Cooper, 2013). The phylogenetic signal therefore estimates
229 the extent to which associations between traits reflect their shared evolutionary history
230 (Freckleton et al., 2002), i.e. the degree of similarity among closely related species compared to
231 distantly related species. We ran bivariate linear regressions of \log_{10} -transformed TL (response
232 variable) and chromosome length (18 species), genome size (32 species), PC1, \log_{10} -transformed
233 maximum lifespan, and \log_{10} -transformed body mass (58 species), respectively. We also tested
234 associations between TL and the life-history traits while accounting for either chromosome
235 length or genome size using phylogenetic multiple regressions (Grafen, 1989). Due to the limited
236 sample sizes, we ran alternative evolutionary models assuming either $\lambda=1$ (BM model), $\lambda=0$ (ST
237 model) or the maximum λ likelihood value of λ (PA model). We then used Akaike’s information
238 criteria corrected for small sample sizes (AIC_c) to determine the best model (Burnham &
239 Anderson, 2002), which is reported in the results. All analyses were performed in R v. 3.6.3 (R
240 Core Team, 2020).

241 **Sensitivity and outlier analyses**

242 We performed sensitivity and outlier analyses within the phylogenetic context to test the
243 robustness of our results to species sampling. We used a phylogenetic leave-one-out deletion
244 analysis implemented in the ‘sensiPhy’ package (Paterno, Penone, & Werner, 2018) to test if any
245 species are strongly affecting the associations. Species were sequentially removed one at a time

246 and the phylogenetic regression refitted using ‘phylolm’ (Tung Ho & Ané, 2014). Highly
247 influential species (outliers resulting in a standardized difference in β -estimates >2 , Paterno et
248 al., 2018) were then excluded and the regressions analyses rerun to obtain more robust
249 phylogenetic regression estimates. We used a jackknifing method randomly removing a
250 proportion of species (from 10 to 50%) and then refitted all regression models described above
251 500 times to estimate sensitivity of β -, λ -, and p -values to changes in sample size (Paterno et al.,
252 2018).

253

254 **RESULTS**

255 **Telomere length evolution**

256 The greatest diversity in early-life TL was found within Passeriformes (6.2-50.5 kb, 25
257 species), whereas the other orders had relatively shorter and less variable TLs (7.1-34.9 kb, 33
258 species, Fig. 1). The ancestral TL of the 58 bird species was inferred, with a wide confidence
259 interval, to be relatively short (12.4 kb, 95% confidence interval (CI)=[-1.1, 25.8], 97.9 Mya)
260 compared to the range of TLs observed in extant species, but close to their average TL (14.0 kb).
261 Phylogenetic signals in life-history traits were close to 1 (Table S4), but for TL $\lambda=0.00$
262 (CI=[0.00, 0.46]).

263 **Associations between telomere length and life-history traits**

264 We found a significant negative association between TL and PC1 across all 58 species
265 (best model: $\beta_{PC1}=-0.003\pm 0.001$ S.E., $p=0.042$, adjusted $R^2=0.055$, $\lambda=0.00$, CI=[0.00, 0.27], Fig.
266 2a), suggestive of shorter TL in slower life-history species. There was also a negative association
267 between TL and maximum lifespan ($\beta_{\log(lifespan)}=-0.230\pm 0.094$ S.E., $p=0.018$, adjusted $R^2=0.080$,
268 $\lambda=0.00$, CI=[0.00, 0.27], Fig. 2b). Thus, a 1% increase in lifespan predicted a 0.23% decrease in

269 TL. We found weak evidence for a negative association between body mass and TL ($\beta_{\log(mass)}=-$
270 0.042 ± 0.026 S.E., $p=0.107$, adjusted $R^2=0.029$, $\lambda=0.00$, CI=[0.00, 0.28], Fig. 2c).

271 We identified up to four phylogenetically significant outliers in the regressions between
272 TL and life-history traits, but their exclusion only led to slight attenuations of the associations
273 (see Figs. S1-S3 in the Supporting Information). These associations were generally robust to
274 smaller sample size effects (mean change in β with 50% species removed was 36-52%) and the
275 phylogenetic association was 0 in most simulations (Figs. S4-S6).

276 **Associations between telomere length, chromosome length and genome size**

277 Genome size positively predicted chromosome length ($\beta_{\log(genome\ size)}=0.024\pm 0.005$ standard
278 error [S.E.], $p<0.001$, adjusted $R^2=0.599$, $\lambda=1.00$, CI=[0.00, 1.00], 18 species, Fig. S7),
279 suggesting that genome size may be used as a proxy for chromosome length in birds.

280 We found weak evidence for a positive association between TL and chromosome length
281 (best model: $\beta_{\log(chromosome\ length)}=1.345\pm 1.029$ S.E., $p=0.210$, $R^2=0.210$ $\lambda=0.00$, CI=[0.00, 1.00],
282 18 species, Fig. 3a). However, the phylogenetic outlier analysis identified one highly influential
283 species, *Ciconia ciconia*, with a disproportional effect on the estimate (resulting in a change of β
284 of 102%, see Paterno et al., 2018 and Fig. S8). Removal of this species revealed a substantial
285 positive association between TL and chromosome length ($\beta_{\log(chromosome\ length)}=2.710\pm 1.106$ S.E.,
286 $p=0.027$, $R^2=0.238$ $\lambda=0.00$, CI=[0.00, 0.99], 17 species, Fig. 3a).

287 There was a marginally significant positive association between TL and genome size
288 (best model: $\beta_{\log(genome\ size)}=1.420\pm 0.826$ S.E., $p=0.096$, adjusted $R^2=0.059$, 32 species, Fig. 3b).
289 We identified three highly influential species, *Aphelocoma coerulescens*, *Parus major*, and
290 *Riparia riparia* (β changes of 50-61%, Fig. S9), the exclusion of which slightly attenuated the fit

291 $(\beta_{\log(\text{genome size})}=1.158\pm 0.742 \text{ S.E.}, p=0.130, R^2=0.049, \lambda=0.00, \text{CI}=[0.00, 0.74], 29 \text{ species, Fig.}$
292 3b).

293 The associations between TL, chromosome length, and genome size were relatively
294 unstable to larger reductions in sample sizes (changes in β with around 50% of the species
295 removed were 45-81%, Fig. S10-S11).

296 **Multiple phylogenetic regressions of life-history and cytogenetic traits**

297 The associations described above between life-history traits and TL, and between
298 cytogenetic traits and TL were slightly or substantially attenuated when included in phylogenetic
299 multiple regressions (Table S4). Mass, lifespan, and PC1 were only weakly correlated with
300 genome size and chromosome length (Table S5), however, sample size was considerably reduced
301 (by 44% when including genome size [32 species] and 68% when including chromosome length
302 [18 species]).

303

304 **DISCUSSION**

305 In this study, we showed that variation in mean early-life TL was significantly associated
306 with key life-history traits underlying the pace-of-life continuum across 58 bird species. This
307 result is consistent with the hypothesis that TL may be an important mediator of life-history
308 trade-offs between reproduction, somatic maintenance, and cancer risk (Heidinger et al., 2012;
309 Monaghan, 2010; Tian et al., 2018). Furthermore, we found some support for a positive
310 association between TL and mean chromosome length or genome size. This indicates that a
311 component of variation in TL may be constrained by interactions with chromosome length across
312 species (Klegarth & Eisenberg, 2018; Slijepcevic, 2016).

313 Early-life TL was significantly negatively associated with maximum lifespan, but we
314 only found weak evidence for a negative association with body mass (Fig. 2b-c). In mammals,
315 lifespan and mass are strongly negatively associated with mean TL (Gomes et al., 2011; Pepke &
316 Eisenberg, 2021), suggesting that these are general relationships across tetrapods. In birds, body
317 size is constrained by adaptations to flight (Tobalske, 2016) and body masses within our study
318 vary by almost 3 orders of magnitude compared to 7 orders of magnitude within the study of
319 mammalian TL (Gomes et al., 2011), which may explain the weaker association between TL and
320 mass in birds. However, there is still a large variation in TL particularly among small species,
321 within both mammals and birds. This variation may be explained by the larger diversity of
322 mechanisms evolved to deal with cancer defenses or cellular senescence within smaller bodied
323 species (Risques & Promislow, 2018; Seluanov, Gladyshev, Vijg, & Gorbunova, 2018). That
324 larger and longer-lived species of tetrapods have short telomeres, has been interpreted as an anti-
325 cancer mechanism, limiting the risk of accumulating oncogenic mutations through replicative
326 cell senescence (Campisi, 2001; Gorbunova et al., 2014; Pepke & Eisenberg, 2021). Research on
327 cancer prevalence in wild bird species is very limited (Møller, Erritzøe, & Soler, 2017;
328 Pesavento, Agnew, Keel, & Woolard, 2018). Boddy et al. (2020) found a positive relationship
329 between litter size and cancer prevalence in 37 mammal species. They suggested that the faster
330 pace-of-life associated with larger litter sizes, exposed fast life-history species to higher cancer
331 rates by allocating more resources to offspring quantity than somatic maintenance. In our study,
332 fast-lived species had a low PC1 score (i.e. short generation time and lifespan, large clutch size,
333 and to some extent a small body mass, Table S3) and long telomeres compared to slow-lived
334 species with a high PC1 score and short telomeres (Fig. 2a). If fast life-history bird species also
335 have higher cancer rates, as suggested by Møller et al. (2017), these observations are consistent

336 with the fact that longer telomeres are associated with increased cancer prevalence across species
337 (in mammals, Pepke & Eisenberg, 2021). Thus, TL may have evolved to be longer to avoid the
338 greater risk of critically short telomeres faced by species with accelerated TL shortening due to
339 increased oxidative stress associated with high rates of reproduction (Sudyka, 2019). Selection
340 for longer telomeres may have been further promoted by the lower antioxidant capacity or higher
341 levels of oxidative damage found in bird species with a faster pace-of-life (Vágási et al., 2019;
342 Xia & Møller, 2018). Bird species with a slower pace-of-life have also been found to have a
343 lower telomere shortening rate (Dantzer & Fletcher, 2015), which suggests that TL and TL
344 attrition co-vary across species, but this has not yet been shown (Tricola et al., 2018).

345 The sensitivity and outlier analyses indicated that the associations between TL and
346 cytogenetic traits were susceptible to sample size effects (Figs. S8-S11). However, our results
347 suggest an interaction between TL evolution and karyotype evolution. We found that a 1%
348 increase in chromosome length was associated with a 2.7% increase in TL (Fig. 3a). The
349 taxonomic diversity of species exhibiting positive scaling between TL and chromosome length
350 within species (reviewed in the introduction) suggests that this is a highly conserved,
351 fundamental characteristic of telomere biology. The explanation behind the positive correlation
352 between telomere and chromosome lengths, remains unknown, but several molecular
353 mechanisms may be involved (Klegarth & Eisenberg, 2018).

354 Experiments in yeast have shown that if telomeric and centromeric sequences are inserted
355 into plasmids, they become unstable, probably because they are being pulled away from each
356 other during mitosis (Enomoto, Longtine, & Berman, 1994). Slijepcevic (2016) suggested that
357 this telomere–centromere antagonism could explain the correlation between TL and chromosome
358 length observed within some species, i.e. the length of telomeres closer to centromeres is shorter

359 to mitigate interference during mitosis. Furthermore, longer telomeres may be needed to protect
360 longer chromosomes from end denaturation and rearrangements (Pampalona, Soler, Genescà, &
361 Tusell, 2010; Slijepcevic, 1998). Supporting the connection between TL and chromosome size,
362 Pontremoli et al. (2018) found that positive selection on genes implicated in telomere
363 homeostasis among mammals was related to the number of chromosome arms. Given that
364 genome size is relatively conserved among mammals (Kapusta et al., 2017), the positive
365 selection at these genes is likely driven by chromosome size and these genes might help calibrate
366 specific telomeres to the corresponding chromosomes. Assuming causality of the telomere–
367 chromosome length association, more chromosome arms results in multiple short telomeres. This
368 may facilitate chromosomal rearrangements (Murnane, 2012; Sánchez-Guillén et al., 2015;
369 Slijepcevic, 1998), but also lead to a higher recombination rate (Pardo-Manuel de Villena &
370 Sapienza, 2001).

371 Among mammals, the association between TL and chromosome length was highly
372 influenced by the karyotypic abnormal Indian Muntjac (Klegarth & Eisenberg, 2018). However,
373 the association remained non-significant after outlier exclusion. This study relied primarily on
374 estimates of adult TL from cultured cell lines (Gomes et al., 2011). Our analysis may have better
375 resolution by including mainly terminal TLs in early life, thereby reducing the effects of
376 differing TL changes through life (Tricola et al., 2018). Consistent with the mammalian
377 sensitivity to the karyotypic abnormal Indian Muntjac, our results were strongly influenced by
378 the outlying white stork, *Ciconia ciconia* (8.8 kb, Fig. 3a), of a genus known to have undergone
379 several chromosomal rearrangements (de Boer & van Brink, 1982, Fig. S7). For instance, *C.*
380 *ciconia* ($2n=72$) probably has many more microchromosomes than the black stork, *C. nigra*
381 ($2n=52$, de Boer & van Brink, 1982), whose mean TL we may then expect to be long, but that is

382 currently unknown. The observation of ultra-long telomeres on some microchromosomes (Nanda
383 et al., 2002) does not conform with the general patterns observed in this study. Since
384 microchromosomes constitutes only around 23% of the avian genome size and are remarkably
385 conserved across most bird species (Burt, 2002; O'Connor et al., 2019), it may be that the
386 patterns reported here primarily retain to the telomere dynamics of macrochromosomes.

387 Within birds, larger genomes have been associated with lower metabolic rate
388 (Vinogradov, 1997), reduced capacity for flight efficiency (Andrews, Mackenzie, & Gregory,
389 2009), and increased body size (Wright et al., 2014). The mechanism underlying these
390 correlations may be acting through a positive relationship between genome size and cell size
391 (Wright et al., 2014). However, if part of the variation in genome size is due to variation in
392 telomere–chromosome length interactions, we suggest that some of these associations may
393 involve adaptations in TL to different life-history strategies, as indicated in this study. For
394 instance, correlations between life-history traits and genome size (Womack, Metz, & Hoke,
395 2019) may involve telomere–chromosome length dynamics.

396 We found TL to be evolutionary labile across bird species, as exemplified by the large
397 intrageneric variation within *Aphelocoma*, *Larus*, *Tachycineta*, and *Thalassarche*, suggesting
398 recent evolutionary change in TL (Fig. 1). Reconstructing the evolutionary history of TL changes
399 within recent radiations of closely related species that represent independent replicated branching
400 events, may elucidate adaptations underlying shifts in TL during speciation (Baird, 2018). As
401 species progress through series of changes in species ecology and life-history (Pepke, Irestedt,
402 Fjeldså, Rahbek, & Jönsson, 2019), associated changes in telomere biology may be observed
403 within taxonomically more densely sampled clades (Canestrelli et al., 2020; Dupoué et al.,
404 2017).

405 Our results indicate that some of the variation in early-life TL in birds arises through
406 interactions with chromosome length, which may constrain the evolution of TL. Future cross-
407 species studies attentive to the specificity of TL at different chromosome arms (Miga et al.,
408 2020; Poon & Lansdorp, 2001), in particular of microchromosomes, may resolve the details of
409 this interaction. Whether this effect has implications for telomere loss and the variation in
410 senescence pattern across species remains unknown. However, mean TL also co-evolved with
411 key life-history traits suggesting that the adaptive significance of TL may be as an important
412 mediator of life-history trade-offs between investment in reproduction and somatic maintenance.

413

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421

422 **AUTHOR CONTRIBUTIONS**

423 MLP and DTAE conceived the ideas. MLP compiled and analyzed data and wrote the
424 manuscript with contributions from all authors.

425

426 **DATA ACCESSIBILITY**

427 All data is available from Table S1 in the Supporting information and from BirdTree.org, and it
428 will be submitted to an open access data repository.

429

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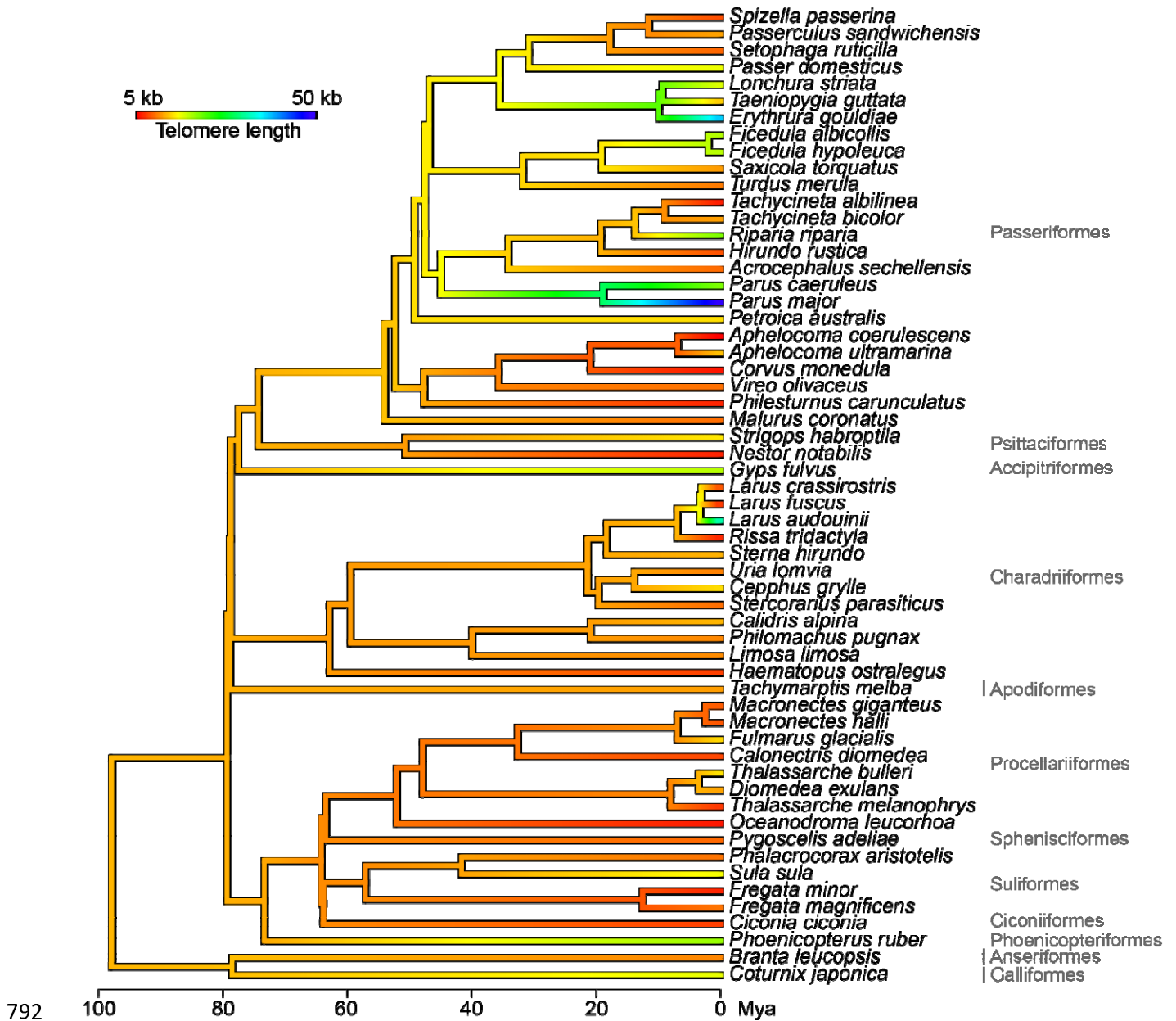
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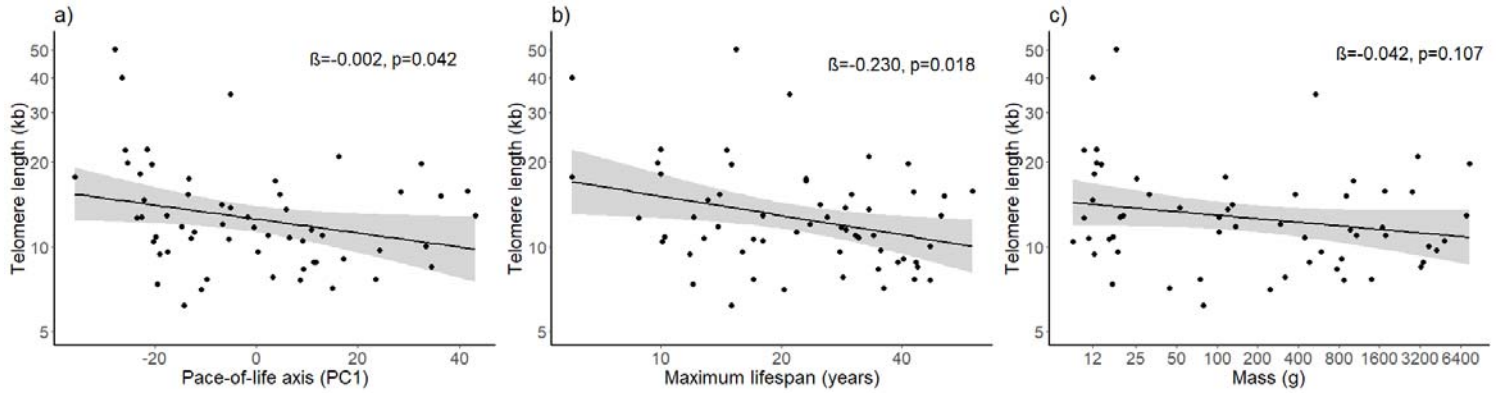
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791 **FIGURES**



792
793 **Fig. 1:** Calibrated maximum clade credibility tree illustrating the evolution of early-life telomere
794 length in 58 bird species using a continuous color gradient (red: short telomeres; blue: long
795 telomeres). Orders are shown on the right, and the timescale is in million years ago (Mya).

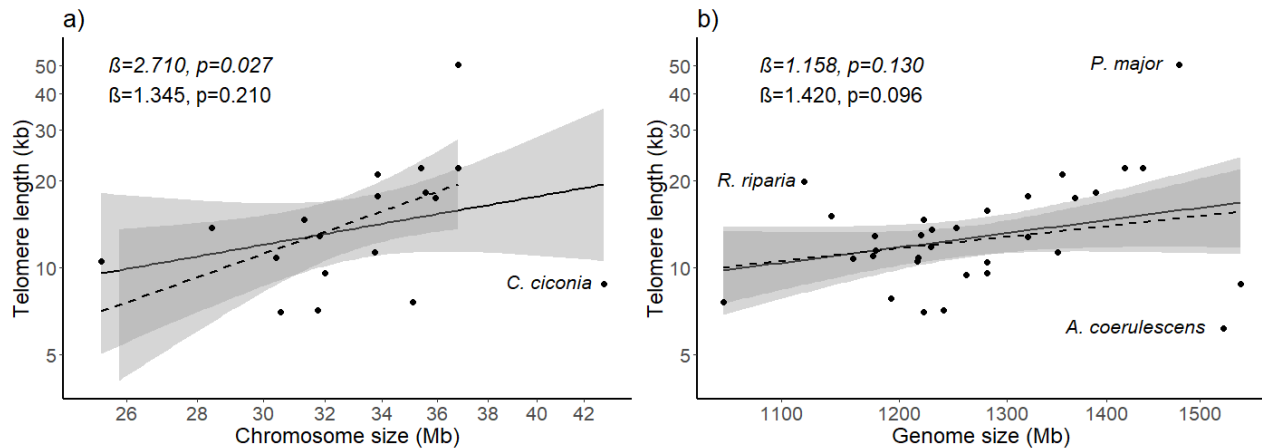
796



797 **Fig. 2:** Associations between \log_{10} -transformed early-life telomere length (kb) and a) PC1 scores
 798 from a phylogenetic principal component analysis reflecting the slow-fast continuum of life-
 799 history trait variation across 58 bird species, b) \log_{10} -transformed maximum lifespan in years,
 800 and c) \log_{10} -transformed body mass in g. Scatter plots do not depict phylogenetic corrections.
 801 Phylogenetic regression lines and their associated statistics are shown. Grey shadings correspond
 802 to 95% confidence intervals.

803

804



805 **Fig. 3:** Associations between average \log_{10} -transformed early-life telomere length (kb) and a)
 806 chromosome size (Mb) for 18 bird species and b) genome size (Mb) for 32 bird species. Scatter
 807 plots do not show phylogenetic corrections. Phylogenetic regression lines and their associated
 808 statistics are shown (solid lines). The phylogenetically identified outliers *Ciconia Ciconia* (a),
 809 and *Aphelocoma coerulescens*, *Parus major*, and *Riparia riparia* (b) are excluded from the
 810 regression analyses shown in italics and with dashed regression lines. Grey shadings correspond
 811 to 95% confidence intervals.