1 **Correlation in telomere lengths between feathers and blood cells in**

2 **pied flycatchers**

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11   **Abstract.** The length of telomeres (*i.e.* the protective non-coding DNA repeat sequences

12   capping the end of eukaryotic chromosomes) is drawing an increasing attention in ecology

13   and evolution as a biomarker of individual state and fate. Bird erythrocytes are nucleated and

14   telomere measurements using blood derived DNA has become the gold standard in avian

15   biology. However, blood sampling is not trivial and not achievable under all field conditions.

16   We investigated whether feather DNA could be used as an alternative or complementary

17   approach to blood DNA when assessing telomere length with the quantitative PCR method.

18   Indeed, investigating telomere length in different tissues may provide more detailed

19   information regarding both the determinants and the importance of telomere length for avian

20   life histories. We collected tertiary feathers on the same day as a blood sample in adult and

21   12-day-old nestling pied flycatchers (*Ficedula hypoleuca*). Our results show a positive but

22   moderate relationship between telomere length measured using DNA derived from blood and

23   feather samples. This relationship was stronger in nestlings than in adults. Nestlings had

24   longer telomeres in blood than in feathers while no significant difference was observed in

25   adults. Hence, our study demonstrates that feathers can provide a complementary approach

26   to blood for telomere measurements in wild birds, and we discuss further methodological

27   considerations when using feathers for telomere measures. Telomeres seem to show faster

28   erosion with age in blood than feathers, which may account for the lower correlation in

29   telomere lengths between the two tissues in adults.

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31   **Keywords** telomeres, ageing, conservation physiology, blood sampling, stress

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Introduction

Ecologists and evolutionary biologists are showing a growing interest for the study of telomeres since telomere length or rate of erosion might provide an integrative view on individual life history trajectories by linking together development, reproduction and life expectancy (Young 2018). Telomeres are highly conserved non-coding DNA repeat sequences that cap the ends of eukaryotic chromosomes, and their main role is to protect genome integrity (Blackburn 2005). Because DNA polymerase is unable to copy the ends of chromosomes during replication (the so-called 'end replication problem'), one key aspect of telomere biology in somatic tissues is that they are shortening at each cell division (Blackburn 2005). Additionally, oxidative stress can damage telomeres, thereby accelerating their shortening (Reichert and Stier 2017). When telomeres reach a critical size they trigger cell replicative senescence (Blackburn 2005). Hence, telomere dynamics has been suggested to mirror organismal ageing, and in turn individual state, as well as to predict, at least partially, individual life expectancy or fate (Wilbourn et al. 2018, Young 2018). Accordingly, evidence is accumulating in captive and wild bird populations from various species that individuals with long telomeres have higher survival and are longer lived (Haussmann et al. 2005, Wilbourn et al. 2018, Eastwood et al. 2019). Because telomere shortening can be hasten in response to harsh environmental conditions (Angelier et al. 2018, Chatelain et al. 2020), telomere length is not only shaped by genetic factors but also by environmental factors (Voillemot et al. 2012, Stier et al. 2016, Kärkkäinen et al. 2019). Hence, telomere dynamics might also account, to some degree, for the links between development, lifestyle and lifespan (Monaghan 2010).

In the last decade, there have been numerous progresses in the measurement of telomere length, one important step being the development of a quantitative polymerase

56 chain reaction (qPCR)-based method that allow specific amplification of the telomere

57 sequence (Cawthon 2002, Criscuolo et al. 2009). This technique has the advantage of allowing

58 a fast quantification of telomere length from small amount of DNA, and is less sensitive to

59 DNA integrity (Aviv et al. 2011). Blood derived DNA is the gold standard in avian studies of

60 telomeres whatever the method retained for telomere quantification: telomere terminal

61 restriction fragment (TRF) or qPCR (Stier et al. 2015). Although bird erythrocytes are nucleated

62 (Stier et al. 2015), which provide a reliable source of DNA, blood sampling is not trivial and not

63 achievable under all field conditions (Bush et al. 2005). Drawing blood requires a well-trained

64 and competent person, and this approach might not be suitable for instance for endangered

65 species. Furthermore, blood samples are often best-stored frozen (-20°C to -80°C) which

66 requires field-based infrastructures (Reichert et al. 2017). To circumvent those problems,

67 there are growing efforts to develop and validate alternative sampling techniques (Bush et al.

68 2005). Accordingly, Taberlet and Bouvet (1991) have already shown in birds that a single

69 plucked feather could be an alternative to blood as a source of DNA for genetic studies and

70 molecular sexing. Feathers have the advantage to be easily and quickly collected, to be stored

71 at room temperature in a dry, dark place, and that one feather can yield enough DNA for PCR

72 measurement (see also McDonald and Griffith 2011, and Katzner et al. 2012 for an exhaustive

73 discussion of pros and cons of feather use in ornithological studies). Feather sampling is also

74 less invasive than blood sampling, in turn being often a non-regulated procedure. Currently,

75 we are aware of only one published study using feathers for telomere analysis, which

76 demonstrated a positive relationship between parental age and chick telomere length in a

77 long-lived seabird species (Dupont et al. 2018). However, there is currently no information on

78 whether telomere length measured from feather derived DNA provides similar results than

79 blood derived DNA. Previous studies in mammals have shown correlation in telomere length

80 among DNA samples from blood cells and different tissues (Takubo et al. 2002, Okuda et al.

81 2002, Benetos et al. 2011, Daniali et al. 2013, Laubenthal et al. 2016). In non-model

82 vertebrates, one study in zebra finch (*Taeniopygia guttata*) and one in painted dragon

83 (*Ctenophorus pictus*) showed that telomere length from blood cells significantly correlates

84 with telomere length in some tissues, but not all (Reichert et al. 2013, Rollings et al. 2019, but

85 see Parolini et al. 2019 for lack of relationship between different somatic tissues excluding

86 blood). Noteworthy, it has also been suggested that the correlation in telomere length

87 between tissues might depend on the life-stage at sampling (Schmidt et al. 2016), with a

88 weakening correlation between tissues with increasing age. Currently we are lacking

89 information on correlation in telomere length between blood and feather samples, and

90 whether this correlation changes across life-stages (*e.g.* nestlings versus adulthood).

91   Hence, the aim of this study was to determine whether feather sampling could be used

92 as an alternative or complementary approach to blood sampling when assessing telomere

93 length with the qPCR method. To address this objective, we collected feather and blood

94 samples from adult and 12-day-old nestling pied flycatchers (*Ficedula hypoleuca*) and tested

95 for relationships between telomere length derived from blood and feather samples.

96 **Materials and methods**

97 Study species and study site

98 The pied flycatcher is a small European passerine that produces one clutch per year consisting

99 of 5 to 7 eggs. Nestlings leave their nest 14 to 16 days after hatching (Lundberg and Alatalo

100 1992). Fieldwork was performed in summer 2018 in a population of pied flycatchers breeding

101 in Ruissalo Island (Finland).

Tissue sampling and genomic DNA extraction

We plucked the second innermost tertiary feather from each wing of 24 adults (12 females and 12 males) and 24 12-day-old chicks on the same day as we drew blood from the brachial vein with non-heparinized capillary tubes. We chose to pluck tertiary rather than body contour feathers to ensure comparison of the same feathers across individuals. Since previous studies using feathers as a source of DNA stored them dry and protected from light in an envelope at ambient temperature (*e.g.* Harvey et al. 2006) and that such conditions are likely to reflect field conditions in many cases where blood sampling is not an option, we chose to store our feather samples under the same conditions until later analyses in the laboratory. Blood samples were diluted into PBS and stored in cold bags while in the field before being stored at −80°C at the end of the day until later analyses. Genomic DNA was extracted from whole blood samples and feather samples (tip of the calamus from each feather) within three months after collection using salt extraction alcohol precipitation method (Aljanabi and Martinez 1997). Extracted DNA was diluted in elution buffer BE (Macherey-Nagel, Düren, Germany) for DNA preservation. DNA quantification and quality check (*i.e.* based on the 260/280 ratio, see Table 1) was performed using ND-1000-Spectrophotometer (NanoDrop Technologies, Wilmington, USA). DNA integrity was assessed using gel electrophoresis (50 ng of DNA, 0.8 % agarose gel at 100 mV for 60 min) and DNA staining with Midori Green (see ESM1 for a representative gel).

Quantitative PCR measurements of telomere length

Telomere length was quantified using a real-time quantitative PCR (qPCR) developed to measure relative telomere length in humans (Cawthon 2002) and later validated in birds (Criscuolo et al. 2009). This technique estimates relative telomere length by determining the ratio (T/S) of

125 telomere repeat copy number (T) to a single copy gene (SCG). Here, we used RAG1 as a SCG

126 (verified as single copy using a BLAST analysis on the collared flycatcher *Ficedula albicollis*)

127 genome.   Forward   and   reverse   telomere   primers   were   5'-

128 CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3' (Tel-1b)   and   5'-

129 GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3' (Tel-2b), respectively, and forward and

130 reverse   RAG1   primers   were   5'-GCAGATGAACTGGAGGCTATAA-3'   and   5'-

131 CAGCTGAGAAACGTGTTGATTC-3'. Both primers were used at a concentration of 200 nM. qPCR

132 primers also amplify non-telomeric (TTAGGG)n sequences, such as interstitial telomeric

133 sequences which are common in birds (Foote et al. 2013). However, we showed previously a

134 strong correlation ($r = 0.74$, p = 0.004) between telomere lengths measured with qPCR and *in-gel*

135 TRF (telomere terminal restriction fragment method, which measures only the terminal telomeric

136 sequences), therefore validating the use of qPCR in the pied flycatcher (Kärkkäinen et al. 2019).

137 For the qPCR assay, we used 5 ng of DNA per reaction in a total volume of 10 µl (8 µl of master

138 mix + 2 µl of DNA). The master mix contained 0.1 µl of each primer, 2.8 µl of water and 5 µl of

139 SensiFAST SYBR Lo-ROX master mix (Bioline, London, UK) per reaction. Our qPCR conditions

140 were: an initial denaturation (1 cycle of 3 minutes at 95°C), 40 cycles with first step of 10

141 seconds at 95°C, second step of 15 seconds at 58°C and third step of 10 seconds at 72°C, with

142 melting curve analysis in the end. qPCR measurements were performed using QuantStudio™

143 12K Flex Real-Time PCR System (Thermo Fisher) using 384-well plates. Telomere and RAG1

144 amplifications were performed in triplicates adjacent to each other on the same plate and

145 each plate contained three internal standards and one negative control. We used LinRegPCR

146 (Ruijter et al. 2009) to determine the baseline fluorescence, the qPCR efficiencies (efficiencies

147 per age class and sample type based on well-specific efficiencies are reported in Table 1) and

148 the quantification cycle (Cq) values. Telomere lengths were calculated based on plate-specific

149 efficiencies (average [min, max] efficiencies for telomere and SCG reactions were,

150 respectively, 2.07 [1.99, 2.15] and 1.98 [1.93, 2.01]) using the mathematical model presented

151 in Pfaffl et al. (2001). Technical repeatability based on triplicate measurements of telomere

152 length was 0.857 (95% CI [0.830, 0.877], $p < 0.001$), and inter-plate repeatability based on

153 samples measured on more than one plate was 0.882 (95% CI [0.808, 0.930], $p < 0.001$).

154 Repeatability is considered to be an informative estimate of measurement error, while the

155 traditionally used coefficient of variation (CV) is deemed to be misleading for qPCR derived

156 telomere data (Verhulst et al. 2015).

157 Statistical analyses

158 First, we analysed the relationship between blood and feather telomere length using a general

159 linear model (GLM) with feather telomere length as the dependent variable and blood

160 telomere length, age class (chicks *vs.* adults), sex and the interactions between feather

161 telomere length and age class as fixed factors. As a complementary approach, we also

162 analysed the between-tissue repeatability in telomere length using the *rptR* package in *R*

163 (Stoffel et al. 2017), with telomere length as the dependent variable, tissue as a fixed factor

164 to account for between-tissues differences in telomere length and bird ID as the random

165 effect. We ran this model on the overall dataset and then subsequently for chicks and adults

166 separately. Finally, we analysed the differences between tissues and age classes using a

167 generalized estimating equation (GEE) following a Gaussian distribution, with telomere length

168 as the dependent variable, age class, tissue, sex and their interactions as fixed factors, as well

169 as bird ID as a random effect. Non-significant interactions were removed from final models

170 and p-values < 0.05 were considered as significant. Statistical analyses were conducted using

171 *SPSS* 24.0 and *R* 3.5.3.

172 **Results**

173 DNA integrity gels revealed that while blood-derived DNA was well preserved, feather-derived

174 DNA from both adult and chicks showed moderate signs of degradation (see ESM Figure 1).

175 Feather telomere length was significantly associated with blood telomere length (p=0.001;

176 Table 2). The non-significant (p = 0.23) interaction between age class and feather telomere

177 length indicates that the relationship between blood and feather telomere length does not

178 differ significantly between 12-day-old chicks and adults (Figure 1A, Table 2). Yet, while the

179 between-tissue repeatability was overall significant and of medium effect size (0.43, CI [0.17,

180 0.62], p < 0.001 Figure 1B), the between-tissue repeatability in chicks was significant and of

181 large effect size (0.55, [0.23, 0.77], p = 0.002; Figure 1B) while adults only showed a non-

182 significant trend associated with a small effect size (0.26, [0.00, 0.60], p = 0.09; Figure 1B).

183 Overall telomere length was influenced by the age class of the bird and the tissue type, but

184 also by the interaction between age class and tissue (Figure 1C, Table 3). Post-hoc tests

185 revealed that chicks had longer telomeres in blood than feathers, while there was no

186 significant difference between adult blood and feather telomere length (Figure 1C). Chicks

187 also had longer telomeres than adults in blood cells, while the difference between chick

188 feather telomere length and adult telomere length did not reach statistical significance (Figure

189 1C).

190 **Discussion**

191 Despite signs of moderate DNA degradation in feather samples, we found that telomere

192 length measured from feather samples is significantly positively correlated with telomere

193   length measured in blood cells, thereby suggesting that such relationship is robust.

194   Relationship in telomere length across various tissues has been well characterised in humans

195   and other mammals (Takubo et al. 2002, Okuda et al. 2002, Benetos et al. 2011, Daniali et al.

196   2013, Laubenthal et al. 2016), but only to a lesser extent in birds (Reichert et al. 2013, Schmidt

197   et al. 2016, Parolini et al. 2019). Previous studies in birds have investigated telomere

198   correlation between erythrocytes, bone marrow, spleen, muscle, heart, liver and brain in adult

199   zebra finches (Reichert et al. 2013), blood, heart and liver in embryonic and juvenile Franklins'

200   gulls, *Leucophaeus pipixcan* (Schmidt et al. 2016) and heart, pectoral muscle, liver and brain

201   in embryonic yellow-legged gulls, *Larus michahellis* (Parolini et al. 2019). These studies

202   showed that telomere length from blood samples correlate with telomere length in several

203   other tissues while correlations among other tissues were less evident. Here, we add to these

204   studies information about the relationship between telomere length measured in blood and

205   feather samples, in both nestling and adult birds. Our results provide additional support to the

206   idea that telomere length from blood, which has become the golden standard in avian biology,

207   could be a suitable proxy of telomere length measured in other tissues, such as feathers.

208   However, telomeres are likely to shorten at different rates in different tissues, and thus

209   the correlation in telomere length between tissues is likely to decrease with increasing age.

210   For example, blood telomere length was found to significantly correlate with heart and muscle

211   telomere lengths (r = 0.47 and r = 0.42, respectively) at late-stage embryo but not at the end

212   of the chick post-natal development in the Franklin's gull (Schmidt et al. 2016). Similar results

213   were found in humans: comparison in telomere length across various tissues show strong

214   correlations in new-borns (r = 0.89 to 0.97; Okuda et al. 2002) and moderate to strong

215   correlations among tissues collected in adult subjects (r = 0.38 to 0.84; Takubo et al. 2002,

216   Daniali et al. 2013). Accordingly, in the pied flycatcher we found a stronger correlation

217 between blood and feather telomere length in nestlings than adults. Although this result

218 supports the idea that telomere correlation between tissues weakens with increasing age,

219 three alternative hypotheses may also at least partially account for this result. Firstly, feathers

220 are vascularised during their growth only. Therefore, some blood could be enclosed in the

221 rachis when feathers are collected during their growth (*i.e.* nestling stage or moulting period

222 in adults). Since, feathers were collected at the end of the growth in nestlings, but outside the

223 moulting period in adults, it is not excluded that some blood DNA was extracted from the

224 feathers in nestlings, which in turn could lead to higher correlation in nestlings than adults.

225 However, we did not notice the typical coloration of red blood cells during DNA extraction

226 from feather tips. Secondly, since in our study the time period elapsed between feather

227 formation and blood sampling was much greater in adults (*i.e.* several months between pre-

228 migration moult and chick-rearing) than in chicks (*i.e.* feathers still growing at the time of

229 blood sampling), this could also lead to a lower correlation between blood and feather in

230 adults than nestlings. Finally, we have to note that DNA extraction from adult feathers gave

231 relatively low amount of DNA of moderate quality (Table 1), which could also contribute to

232 explain the lower correlation we obtained here for adult birds. Noteworthy, this difference in

233 correlation does not seem to be related to DNA degradation as both adult and nestling feather

234 samples showed moderate signs of degradation (ESM1), and qPCR has been suggested to be

235 less sensitive than other methods to DNA degradation (Aviv et al. 2011). Hence, future studies

236 are needed to test whether alternative storage methods could help in obtaining high-quality

237 DNA from feather samples, and whether this might affect the magnitude of the correlation

238 found here between blood and feather.

239   In the present study, we also found that nestlings had longer telomeres in blood than

240 in feathers while telomere lengths in adults were similar in both tissues. Shorter telomeres in

241 nestling feathers than in blood may be caused by faster shortening during post-natal

242 development (*i.e.* before nestlings' day 12 when the first feathers are produced) or

243 alternatively, telomere length in feather might be initially shorter than telomere length in

244 blood cells. Notably, the expected age difference in telomere length between chicks and

245 adults was pronounced in blood cells, while not being strong enough to reach statistical

246 significance in feathers. Yet, again, we have to be careful regarding the results for adult

247 feathers due to the moderate quality of the DNA extracted from this sample type. Additionally,

248 comparing telomere length between feathers and blood using a technique allowing the

249 characterization of telomere length distribution (*i.e.* TRF) would allow to better explore the

250 similarities and differences in telomere length and dynamics between these two tissues.

251   While considering our results as a whole, it appears that measuring telomere length

252 from feathers should be considered more as a complementary approach (*i.e.* in addition to

253 blood or when blood sampling is not possible) rather than a universal alternative to blood.

254 Indeed, telomere length and age-related changes differed between tissues, correlations

255 between tissues were only moderate, and DNA from feathers showed moderate signs of

256 degradation. Yet, the use of feathers to measure telomere length could provide three main

257 advantages. Firstly, just as blood it is a proliferative tissue with feathers being regularly re-

258 grown during a bird lifetime, but unlike blood its proliferation is restricted to short and well-

259 defined time periods (*i.e.* nestling period and pre- or post-breeding moulting periods at

260 adulthood for most bird species). Hence, feather sampling at particular time periods, such as

261 fledgling or post-breeding moult, might help collecting standardised measurements of

262 telomere length and, in turn, help in reducing unwanted variance when exploring inter-

263 individual variation in telomere length. Secondly, because detectable amounts of stress

264 hormone (*i.e.* corticosterone; Bortolotti et al. 2008) and pollutants (Jaspers et al. 2019) can be

265 deposited in growing feathers, this tissue is increasingly used for long-term, integrated

266 measures of avian stress physiology and exposure to pollutants. Thus, coupling the measures

267 of corticosterone and pollutants together with measures of telomere length within the same

268 feathers will allow truly integrative studies based on a minimally invasive sampling approach.

269 Finally, conversely, to blood sampling, feather collection does not require a large amount of

270 training and competences and feathers can be collected from living or dead birds. However,

271 long-term storage might affect DNA integrity and therefore its effects on telomere length

272 should be further investigated.

273   To conclude, the present finding of a positive and significant correlation between

274 telomere length measured in blood and feathers is important for three reasons. Firstly, it

275 demonstrates a significant relationship in telomere length between two different tissues in

276 wild animals. Secondly, it opens the opportunity to measure telomere length in more than

277 one single tissue type in a minimally invasive manner, which is important to evaluate effects

278 at the whole-organism level, and potential tissue-specific effects. Finally, it opens the

279 opportunity of using feathers as an alternative approach, at least in chicks, for measuring

280 telomeres when sampling/storing blood is not possible.

288   **Data availability.** Dataset used in this study will be publicly accessible on Figshare providing

289   acceptance of the manuscript (private link: https://figshare.com/s/dffa03e1e91c2e57dc13).

290   **Author's contribution.** PB initiated this study, TK collected the samples and performed

291   laboratory work, AS analysed the data and supervised TK. All authors wrote the manuscript.

292   **Permits.** Blood sampling was approved by Animal Experiment Board in Finland (authorization

293   license ESAVI/3021/04.10.07/2017).

References

Aljanabi, S. M. and Martinez, I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. - Nucleic Acids Res. 25: 4692–4693.

Angelier, F., Costantini, D., Blévin, P. and Chastel, O. 2018. Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. - Gen. Comp. Endocrinol. 256: 99–111.

Aviv, A., Hunt, S. C., Lin, J., Cao, X., Kimura, M. and Blackburn, E. 2011. Impartial comparative analysis of measurement of leukocyte telomere length/DNA content by Southern blots and qPCR. - Nucleic Acids Res. 39: e134–e134.

Benetos, A., Kimura, M., Labat, C., Buchoff, G. M., Huber, S., Labat, L., Lu, X. and Aviv, A. 2011. A model of canine leukocyte telomere dynamics. - Aging Cell 10: 991–995.

Blackburn, E. H. 2005. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. - FEBS Lett. 579: 859–862.

Bortolotti, G. R., Marchant, T. A., Blas, J. and German, T. 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. - Funct. Ecol. 22: 494–500.

Bush, K. L., Vinsky, M. D., Aldridge, C. L. and Paszkowski, C. A. 2005. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater Sage-Grouse (Centrocercus uropihasianus). - Conserv. Genet. 6: 867–870.

Cawthon, R. M. 2002. Telomere measurement by quantitative PCR. - Nucleic Acids Res. 30: e47–e47.

Chatelain, M., Drobniak, S. M. and Szulkin, M. 2020. The association between stressors and telomeres in non-human vertebrates: a meta-analysis. - Ecol. Lett. 23: 381–398.

Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N. B., Foote, C. G., Griffiths, K., Gault, E. A. and Monaghan, P. 2009. Real-time quantitative PCR assay for measurement of avian telomeres. - J. Avian Biol. 40: 342–347.

Daniali, L., Benetos, A., Susser, E., Kark, J. D., Labat, C., Kimura, M., Desai, K. K., Granick, M. and Aviv, A. 2013. Telomeres shorten at equivalent rates in somatic tissues of adults. - Nat. Commun. 4: 1597.

Dupont, S. M., Barbraud, C., Chastel, O., Delord, K., Ruault, S., Weimerskirch, H. and Angelier, F. 2018. Young parents produce offspring with short telomeres: A study in a long-lived bird, the Black-browed Albatross (Thalassarche melanophrys). - PLOS ONE 13: e0193526.

Eastwood, J. R., Hall, M. L., Teunissen, N., Kingma, S. A., Hidalgo Aranzamendi, N., Fan, M., Roast, M., Verhulst, S. and Peters, A. 2019. Early-life telomere length predicts lifespan and lifetime reproductive success in a wild bird. - Mol. Ecol. 28: 1127–1137.

330 Foote, C., Vleck, D. and Vleck, C. M. 2013. Extent and variability of interstitial telomeric
331   sequences and their effects on estimates of telomere length. - Mol. Ecol. Resour. 13:
332   417–428.

333 Harvey, M. G., Bonter, D. N., Stenzler, L. M. and Lovette, I. J. 2006. A comparison of plucked
334   feathers versus blood samples as DNA sources for molecular sexing. - J. Field
335   Ornithol. 77: 136–140.

336 Haussmann, M. F., Winkler, D. W. and Vleck, C. M. 2005. Longer telomeres associated with
337   higher survival in birds. - Biol. Lett. 1: 212–214.

338 Jaspers, V. L. B., Covaci, A., Herzke, D., Eulaers, I. and Eens, M. 2019. Bird feathers as a
339   biomonitor for environmental pollutants: Prospects and pitfalls. - TrAC Trends Anal.
340   Chem. 118: 223–226.

341 Kärkkäinen, T., Teerikorpi, P., Panda, B., Helle, S., Stier, A. and Laaksonen, T. 2019. Impact of
342   continuous predator threat on telomere dynamics in parent and nestling pied
343   flycatchers. - Oecologia 191: 757–766.

344 Katzner, T. E., Wheeler, M., Negro, J. J., Kapetanakos, Y., DeWoody, J. A., Horvath, M. and
345   Lovette, I. 2012. To pluck or not to pluck: scientific methodologies should be carefully
346   chosen, not 'one size fits all.' - J. Avian Biol. 43: 15–17.

347 Laubenthal, L., Hoelker, M., Frahm, J., Dänicke, S., Gerlach, K., Südekum, K.-H., Sauerwein, H.
348   and Häussler, S. 2016. Short communication: Telomere lengths in different tissues of
349   dairy cows during early and late lactation. - J. Dairy Sci. 99: 4881–4885.

350 Lundberg, A. and Alatalo, R. V. 1992. The Pied Flycatcher. - T. & A.D. Poyser, London.

351 McDonald, P. G. and Griffith, S. C. 2011. To pluck or not to pluck: the hidden ethical and
352   scientific costs of relying on feathers as a primary source of DNA. - J. Avian Biol. 42:
353   197–203.

354 Monaghan, P. 2010. Telomeres and life histories: the long and the short of it. - Ann. N. Y.
355   Acad. Sci. 1206: 130–142.

356 Okuda, K., Bardeguez, A., Gardner, J. P., Rodriguez, P., Ganesh, V., Kimura, M., Skurnick, J.,
357   Awad, G. and Aviv, A. 2002. Telomere Length in the Newborn. - Pediatr. Res. 52: 377–
358   381.

359 Parolini, M., Possenti, C. D., Caprioli, M., Rubolini, D., Romano, A. and Saino, N. 2019. Egg
360   Testosterone Differentially Affects Telomere Length in Somatic Tissues of Yellow-
361   Legged Gull Embryos. - Physiol. Biochem. Zool. PBZ 92: 459–462.

362 Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT–
363   PCR. - Nucleic Acids Res. 29: e45–e45.

364 Reichert, S. and Stier, A. 2017. Does oxidative stress shorten telomeres in vivo? A review. -
365   Biol. Lett. 13: 20170463.

366 Reichert, S., Criscuolo, F., Verinaud, E., Zahn, S. and Massemin, S. 2013. Telomere Length
367   Correlations among Somatic Tissues in Adult Zebra Finches. - PLOS ONE 8: e81496.

368 Reichert, S., Froy, H., Boner, W., Burg, T. M., Daunt, F., Gillespie, R., Griffiths, K., Lewis, S.,
369   Phillips, R. A., Nussey, D. H. and Monaghan, P. 2017. Telomere length measurement
370   by qPCR in birds is affected by storage method of blood samples. - Oecologia: 1–10.

371 Rollings, N., Friesen, C. R., Whittington, C. M., Johansson, R., Shine, R. and Olsson, M. 2019.
372   Sex- And tissue-specific differences in telomere length in a reptile. - Ecol. Evol. 9:
373   6211–6219.

374 Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bakker, O., Hoff, V. D., B, M. J.
375   and Moorman, A. F. M. 2009. Amplification efficiency: linking baseline and bias in the
376   analysis of quantitative PCR data. - Nucleic Acids Res. 37: e45–e45.

377 Schmidt, J. E., Sirman, A. E., Kittilson, J. D., Clark, M. E., Reed, W. L. and Heidinger, B. J. 2016.
378   Telomere correlations during early life in a long-lived seabird. - Exp. Gerontol. 85: 28–
379   32.

380 Stier, A., Reichert, S., Criscuolo, F. and Bize, P. 2015. Red blood cells open promising avenues
381   for longitudinal studies of ageing in laboratory, non-model and wild animals. - Exp.
382   Gerontol. 71: 118–134.

383 Stier, A., Delestrade, A., Bize, P., Zahn, S., Criscuolo, F. and Massemin, S. 2016. Investigating
384   how telomere dynamics, growth and life history covary along an elevation gradient in
385   two passerine species. - J. Avian Biol. 47: 134–140.

386 Stoffel, M. A., Nakagawa, S. and Schielzeth, H. 2017. rptR: repeatability estimation and
387   variance decomposition by generalized linear mixed-effects models. - Methods Ecol.
388   Evol. 8: 1639–1644.

389 Taberlet, P. and Bouvet, J. 1991. A Single Plucked Feather As A Source Of Dna For Bird
390   Genetic-Studies. - Auk 108: 959–960.

391 Takubo, K., Izumiyama-Shimomura, N., Honma, N., Sawabe, M., Arai, T., Kato, M., Oshimura,
392   M. and Nakamura, K.-I. 2002. Telomere lengths are characteristic in each human
393   individual. - Exp. Gerontol. 37: 523–531.

394 Verhulst, S., Susser, E., Factor-Litvak, P. R., Simons, M. J., Benetos, A., Steenstrup, T., Kark, J.
395   D. and Aviv, A. 2015. Commentary: The reliability of telomere length measurements.
396   - Int. J. Epidemiol. 44: 1683–1686.

397 Voillemot, M., Hine, K., Zahn, S., Criscuolo, F., Gustafsson, L., Doligez, B. and Bize, P. 2012.
398   Effects of brood size manipulation and common origin on phenotype and telomere
399   length in nestling collared flycatchers. - BMC Ecol. 12: 17.

400 Wilbourn, R. V., Moatt, J. P., Froy, H., Walling, C. A., Nussey, D. H. and Boonekamp, J. J. 2018.
401   The relationship between telomere length and mortality risk in non-model vertebrate
402   systems: a meta-analysis. - Phil Trans R Soc B 373: 20160447.

403   Young, A. J. 2018. The role of telomeres in the mechanisms and evolution of life-history
404   trade-offs and ageing. - Phil Trans R Soc B 373: 20160452.

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406   **Table 1:** Descriptive (Mean ± SE) information on DNA extracted from blood and feather

407   samples, and associated qPCR efficiencies.

	Chicks day 12		Adults	
	Blood (N=24)	Feather (N=24)	Blood (N=24)	Feather (N=24)
[DNA] ng/ µl	321.42 ± 28.1	677.41 ± 59.0	294.44 ± 42.4	56.86 ± 40.9
260/280 ratio	1.97 ± 0.01	2.16 ± 0.1	1.91 ± 0.01	1.65 ± 0.05
260/230 ratio	2.17 ± 0.03	2.21 ± 0.01	2.19 ± 0.04	1.54 ± 0.19
qPCR efficiency: Telomere	2.04 ± 0.02	2.02 ± 0.02	2.10 ± 0.02	2.07 ± 0.03
qPCR efficiency: SCG	1.96 ± 0.01	2.05 ± 0.03	2.01 ± 0.01	2.01 ± 0.03

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417 <u>**Table 2**</u>**: Summary of the GLM model testing the relationship between telomere length in**

418 **blood and feather samples while accounting for age and sex effects.** Estimates are given for

419 adults (Age class) and for females (Sex). Significant factors are presented in bold and the non-

420 significant interactions have been removed from the final model.

<u>Feather telomere length</u>	Estimate	SE	df	F	p-value
Intercept	1.52	0.35	1, 44	31.4	**< 0.001**
Age class (Adults)	0.10	0.19	1, 44	0.3	0.62
Blood telomere length	0.34	0.10	1, 44	11.5	**0.001**
Sex (Females)	0.19	0.17	1, 44	1.2	0.28
Age class x Feather TL					*ns* (p = 0.23)

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422   **<u>Table 3</u>: Summary of the most parsimonious GEE model testing the effects of tissue type**

423   **(blood *vs.* feathers) and age class (day 12 chicks *vs.* adults) and sex on relative telomere**

424   **length.** Estimates for fixed factors are given for the following levels: Age class = adults, Tissue

425   type = blood, Sex = Female. Significant factors are presented in bold. Non-significant

426   interactions have been removed from the final model, but their p-values from the full model

427   are presented between brackets.

Relative telomere length	Estimate	SE	df	Wald-χ^2	p-value
Intercept	2.63	0.15	1	820.9	**< 0.001**
Age class (Adults)	-0.20	0.19	1	8.9	**0.003**
Tissue (Blood)	0.60	0.16	1	4.6	**0.033**
Sex (Female)	0.18	0.19	1	0.9	0.35
Age class x Tissue	-0.71	0.23	1	9.6	**0.002**
Age class x Sex					*ns* (p = 0.06)
Tissue x Sex					*ns* (p = 0.69)
Age class x Tissue x Sex					*ns* (p = 0.91)

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429   **<u>Figure 1</u>: (A) Relationships between blood and feather relative telomere length according to**

430   **age class.** Dotted lines represent age-specific relationships, and the solid line the overall

431   significant relationship**. (B) Between-tissues adjusted repeatability of relative telomere**

432   **length.** Repeatability estimates are presented for the overall dataset as well as for each age

433   class, and are presented with their 95% confidence interval. Significant repeatability estimates

434   are presented in black and non-significant ones in grey. **(C) Interaction between tissue type**

435   **and age class in determining relative telomere length.** Details of statistical tests are given in

436   the text and in Tables 2 and 3.

437

438   **Figure 1**

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