

Telomere length is reflected by plumage coloration and predicts breeding success in the barn swallow

Marco Parolini^{1*}, Andrea Romano^{1*}, Alessandra Costanzo¹, Lela Khoriauli², Marco Santagostino², Solomon G. Nergadze², Luca Canova³, Diego Rubolini¹, Elena Giulotto², Nicola Saino¹

¹*Department of Biosciences, University of Milan, via Celoria 26, I-20133 Milan, Italy*

²*Department of Biology and Biotechnology, University of Pavia, via Ferrata 1, I-27100 Pavia, Italy*

³*Department of Chemistry, via Taramelli 12, I-27100 Pavia, Italy*

* Correspondence should be addressed to:

Marco Parolini (marco.parolini@unimi.it) and

Andrea Romano (andrea.romano@unimi.it)

Department of Biosciences, via Celoria 26, I-20133 Milan

1 **Abstract**

2 Individuals differ in realized fitness but the genetic/phenotypic traits that underpin such variation are
3 often unknown. Telomere dynamics may be a major source of variation in fitness traits because
4 physiological telomere shortening depends on environmental and genetic factors and can impair
5 individual performance. Here, we showed that, in a socially monogamous, biparental passerine bird, the
6 barn swallow (*Hirundo rustica*), telomere length (TL) of both adult males and females positively predicts
7 seasonal reproductive success, as expected because long telomeres can boost performance. Telomere
8 length was correlated with sexually dimorphic plumage colour traits in both sexes, showing for the first
9 time in any species that coloration reliably reflects TL and may mediate mutual mate choice, leading to
10 the observed positive assortative mating for TL in the barn swallow. Thus, TL appears to be a source of
11 variation in a major fitness trait and may be an ultimate target of mate choice, as individuals of both
12 sexes can use coloration to adaptively choose high-quality mates that possess long telomeres.

13

14 **Keywords:** barn swallow, plumage colour, mate choice, reproductive success, telomeres

15

16 **Introduction**

17 Understanding the causes of individual variation in fitness traits is pivotal to the study of ecological
18 evolutionary processes. Variance in complex life-history traits, like breeding success and survival, likely
19 depends on a large number of so-called ‘quality’ or ‘state’ traits, some of which, however, may have a
20 disproportionate effect on performance owing to their multi-faceted consequences for life-histories.
21 Studies of humans and model organisms have suggested that telomere dynamics are likely candidates as
22 major determinants of variation in individual state because of their pervasive effects on organismal
23 function and crucial processes like senescence [1].

24 Telomeres are nucleoprotein structures located at the termini of eukaryotic chromosomes that contribute
25 to maintaining chromosomal integrity. Telomeric DNA of vertebrates consists of tandem repetitions of
26 the hexamer TTAGGG tightly associated with shelterin complexes, which provide regulation and
27 protection to chromosome ends [2]. Because DNA polymerase cannot fully replicate linear telomeric
28 DNA, in normal somatic cells telomeres undergo shortening at each cell division. When telomere length
29 (TL) reaches a certain threshold, cells enter either replicative senescence or apoptosis, thereby
30 potentially compromising tissue renewal capacity and functioning and, ultimately, performance [1,3,4].
31 For example, in wild bird populations short and/or rapidly shortening telomeres negatively predict life
32 expectancy [5–8], (but see [9]) and reproductive success [10,11].

33 Telomere length and dynamics are partly genetically determined [12,13], (but see [14]). However, the
34 rate at which telomeres shorten partly depends on exposure to diverse forms of stress [1,6,15–17],
35 parasitism [18], social environment and competition effects [19–22], parental effort [20,23,24] and
36 availability of dietary antioxidants [25,26] or oxidative stress [27–29], with unfavourable conditions
37 being associated with reduced telomere length and/or increased telomere erosion.

38 Thus, telomere dynamics have the potential of translating the experience of environmental factors into
39 variation in life-histories [22,23,30]. Because telomere dynamics are a key factor underpinning variation
40 in individual ‘quality’ [31], they may be relevant to sexual selection processes. Sexual selection theory

41 posits that individuals of the sex that invests disproportionately more in reproduction are selected to
42 prefer high-quality individuals as mates because this will accrue them fitness benefits [32]. In species
43 where both sexes appreciably invest in reproduction, however, mate choice is predicted to be mutual,
44 leading to expect positive assortative mating for quality traits. According to Fisherian-handicap models
45 of sexual selection, preference for highly expressed secondary sexual traits that reliably advertise the
46 genetic/phenotypic quality of their bearer can mediate adaptive choice of high-quality mates [32]. The
47 components of variation in individual quality that are the ultimate target of adaptive mate choice,
48 however, are typically difficult to identify. In the present context, we suggest that variation in telomere
49 length may be a major component of variation in quality, and may therefore covary with the expression
50 of sexually dimorphic traits potentially under directional inter-sexual preference.

51 The determinants of telomere dynamics and the role of TL in physiology and pathology of humans and
52 model species has been at the focus of intensive research [31]. However, our knowledge of telomere
53 biology in relation to life-histories in wild organisms is at its infancy, and no study has investigated the
54 relationships between telomeres and sexually selected traits. The aims of the present study of the barn
55 swallow (*Hirundo rustica*), a socially monogamous, semi-colonial passerine bird showing sex-related
56 colour variation [33,34], were thus threefold.

57 First, we tested if seasonal reproductive success covaried with TL. If long telomeres are typical of high-
58 quality individuals, we expected reproductive success to increase with TL.

59 Melanin-based coloration of the ventral plumage region of barn swallows considerably varies among
60 individuals and subspecies [34], ranging from white to chestnut, and also differs on average between the
61 sexes [35], suggesting that coloration may be under sexual selection and that it may potentially reflect
62 inherent individual quality (*sensu* [36]), [37]. In addition, in a previous study of nestlings from the same
63 geographical population it was shown that individuals with darker coloration have longer telomeres [22].

64 Our second aim was therefore to test if ventral plumage coloration reflects TL also in adults, while
65 predicting a positive relationship between colour darkness and TL.

66 Third, we analyzed the relationship between coloration and seasonal reproductive success. Previous
67 studies of other populations have documented a relationship between male coloration and annual
68 breeding success, with darker males producing a larger number of offspring per breeding season, but no
69 within-population-level data from Europe have been published on this relationship [38]. If telomere
70 length correlates with coloration and predicts breeding success, we also expected coloration to predict
71 seasonal reproductive success, with darker individuals showing higher success.

72

73 **Methods**

74 We captured, sexed and marked with numbered metal and colour rings all adult barn swallows
75 breeding at 11 colonies (= farms) in all years between 2010 and 2013. Age of the individuals was
76 assigned thanks to breeding philopatry of our study population and exhaustive capturing of all
77 breeding adults in all years since 2010, according to well established procedures (see ESM 1; see also
78 [39]).

79 In 2013, we collected a blood sample from focal adults for telomere analyses. Blood samples were
80 stored at -80 °C within four hour of collection. Upon capture we also collected ca. 5 contour feathers
81 from the same ventral plumage region from all individuals for later spectrophotometric colour
82 measurement. We assigned adults to nests by observation of individual markings. Nests were
83 regularly inspected to record all breeding events during the breeding season. Barn swallow nestlings
84 usually fledge when approximately 20 days old. Seasonal reproductive success was expressed as the
85 total number of nestlings that were present in the nest at last visit, when nestlings were at least 12
86 days old. This is a good proxy of the number of nestlings fledged because in our population nestling
87 mortality during the second half of the nestling period is very low (<5%, NS unpublished results).

88

89 *Telomere length analysis*

90 The methods for TL analysis of the present sample of individuals are fully reported in Parolini et al.
91 (2015) [40] and in the Electronic Supplementary Material (ESM). Briefly, TL was measured in DNA
92 extracted from blood samples by the monochrome multiplex quantitative PCR method. Telomere
93 length was measured as the ratio (T/S) of the amount of telomeric repeats (T) to that of a single copy
94 gene (S), relative to a reference sample, and expressed as relative telomere length (RTL). As the
95 amount of reference sample DNA was not enough to include it in all the plates, we split the analyses
96 in two different batches and we used two different reference samples. The first group included 22
97 pairs, while the second group included 43 pairs. To solve any differences in the mean RTL between

98 batches due to the dissimilar reference sample we used, RTL data were standardized within each of
99 two groups of pairs by subtracting the group's mean.

100

101 *Spectrometric feather colour analysis*

102 Color of one ventral feather was quantified by recording the reflectance spectra of its distal end (see
103 ESM and [35]). Reflectance data were subsequently processed according to the tetrahedral color
104 space model [41]. Feather color was thus described by three variables: θ and ϕ , which accounts for
105 the 'visible' and the ultraviolet colour component, respectively, and rA , which accounts for saturation.
106 The present method for quantifying coloration provides highly repeatable measures both between
107 repeated measurements of the same feather and between feathers of the same ventral plumage region.
108 Importantly, we have demonstrated that coloration measured according to our present procedure
109 strongly positively reflects coloration as measured directly on the birds [36].

110

111 *Statistical analyses*

112 We relied on Poisson generalized linear models to analyze variation in seasonal reproductive success in
113 relation to RTL and colour. In the models we included age as a continuous covariate because
114 reproductive success can vary with age. Large correlations existed between age and RTL of mates.
115 Because inclusion of highly correlated predictors in linear models can raise multicollinearity issues, we
116 ran separate analyses for males and females. Variation of colour components in relation to RTL was
117 analyzed in Gaussian linear mixed models including breeding pair as a random factor and sex, RTL and
118 their interaction as fixed effects. In addition, we included age as a covariate. The interaction term
119 between sex and RTL, and the effect of age were removed from the models in a single step when
120 statistically non-significant. In all the above models we initially included the random effect of colony.
121 Because log-likelihood tests based on Gaussian linear mixed models did not show any significant
122 contribution ($P > 0.05$ in all cases) of colony, the random effect was removed.

123 The sample consisted of 65 females and their 65 male mates for RTL and seasonal reproductive success.

124 The sample for coloration consisted of 62 females and 65 males (feathers for 3 females were not

125 available).

126

127 **Results**

128 The age of both males and females ranged between 1 and 3 years, was positively correlated between
129 mates ($r = 0.588$, $n = 65$, $P < 0.001$) and did not differ between males and females (paired t -test; $t_{64} =$
130 1.22 , $P = 0.228$). RTL did not differ between males and their mates (paired t -test; $t_{64} = 1.38$, $P = 0.173$)
131 and did not vary with age both in males ($r = 0.01$, $n = 65$, $P = 0.969$) and females ($r = -0.04$, $n = 65$, $P =$
132 0.769).

133

134 *Relative telomere length and reproductive success*

135 Seasonal reproductive success increased with RTL in both sexes (Table 1; Fig. 1). An increase in RTL
136 by 2 standard deviations could be estimated to translate into an increase in seasonal reproductive success
137 by 1.2 offspring for males and 1.3 offspring for females, corresponding to 24% (males) or 25% (females)
138 of the mean observed seasonal reproductive success. Breeding success increased with age, as expected
139 (Table 1).

140

141 *Relative telomere length and coloration*

142 Males had darker 'visible' ventral plumage coloration (θ : paired t -test; $t_{61} = 4.46$, $P < 0.001$) and larger
143 ventral plumage saturation (rA : $t_{61} = 3.68$, $P < 0.001$), and their UV coloration differed from that of
144 females (ϕ : $t_{61} = 3.72$, $P < 0.001$) (Fig. 1S). Because none of the colour components significantly varied
145 with age (r values always associated with $P > 0.21$), these analyses were not influenced by age effects.

146 The relationships of ventral plumage colour components with RTL varied in a complex way between
147 the sexes. Darkness of the 'visible' coloration (as reflected by decreasing values of θ), increased with
148 RTL in males whereas it was not significantly associated with RTL in females, yielding a significant sex
149 by RTL interaction effect (Table 2; Fig. 2). The values of the ϕ (UV) colour component increased with
150 RTL independently of sex (Table 2; Fig. 2). In addition, rA significantly decreased with RTL in females

151 while it non-significantly increased with RTL in males, again yielding a significant sex by RTL
152 interaction effect (Table 2; Fig. 2). Thus, coloration covaried with RTL, but in a sex-dependent way.

153

154 *Coloration and seasonal reproductive success*

155 Seasonal reproductive success differently covaried with ventral plumage coloration in either sex. Darker
156 males had significantly larger breeding success whereas no significant relationship existed between
157 'visible' coloration and reproductive success in females (Table 3; Fig. 2S). In addition, reproductive
158 success of males but not females increased with increasing UV reflectance whereas it did not covary
159 with colour rA (Table 3; Fig. 2S).

160

161 **Discussion**

162 Telomeres have the potential to contribute a major component of variation in life-history traits of
163 eukaryotic organisms under both physiological and pathological conditions because their shortening
164 dynamics are sensitive to environmental as well as genetic background, and have pervasive
165 consequences for organismal function [1]. In fact, the first main finding of our study was that seasonal
166 reproductive success of both male and female barn swallows positively covaried with TL. These results
167 are by necessity correlational, because experimental manipulation of telomere length can hardly be
168 devised in the wild (but see [42] for lab experimental approach), and therefore do not conclusively
169 demonstrate causality. The relationship between breeding success and TL, however, was in the expected
170 direction because long telomeres are predicted to enhance general individual performance, and thus
171 seasonal reproductive success. These relationships were observed while controlling for the potentially
172 confounding effect of individual age. Since positive assortative mating for telomere length exists in the
173 present population of barn swallows ($r = 0.384$, $n = 65$, $P = 0.002$; Khoriauli *et al.*, submitted), a
174 proximate explanation for a causal positive relationship between TL and seasonal reproductive success
175 is that high-quality males with long telomeres mate with relatively fecund females which also have long
176 telomeres, and/or that individuals with relatively long telomeres perform better at parental duties.

177 Positive assortative mating for TL (Khoriauli *et al.*, submitted) suggested the existence of traits that
178 reliably signal TL in both sexes, and that assortative mating results from mutual preference for high-
179 quality mates, as expected in a species with extensive biparental reproductive investment. Here, we
180 demonstrated for the first time in any species that TL covaries with sexually dimorphic coloration in
181 both sexes and may therefore mediate mutual sexual choice leading to assortative mating. Interestingly,
182 the covariation between TL and individual components of sexually dimorphic coloration differed
183 between the sexes and in females existed only for the UV component and for colour saturation. This
184 highlights the importance of considering all colour components in studies of communication in this and
185 potentially also in the majority of the other bird species, which perceive UV wavelengths.

186 A relationship between coloration and RTL was previously documented in nestlings from the same
187 population [22]. Differently from the present results, however, the relationship was not sex-dependent
188 (our unpubl. results). Because nestling barn swallows show null or small colour sexual dimorphism
189 [22,37] whereas adult males strongly differ in average coloration from females, the sex-dependent
190 association between coloration and RTL appears to be established only in adulthood, when sexual
191 differences in coloration are also established. The mechanisms that link telomere length to melanin-
192 based plumage coloration are open to speculation. A link between melanogenesis and telomere dynamics
193 may arise because the activity of telomerase, an enzyme that functions to restore telomere length [43],
194 affects the expression of tyrosinase, which is involved in early melanin biosynthetic pathways [44]. A
195 general physiological link between melanogenesis and telomere dynamics may also arise via the
196 pleiotropic effects of genes of the melanocortin system, which control melanogenesis and intervene in a
197 number of physiological functions including response to diverse forms of stress and hence telomere
198 dynamics [45].

199 The barn swallow is widely distributed across the Holarctic region and morphological variation exists
200 across its range in sexually dimorphic traits as well as in sexual dimorphism [33,34]. In addition, sexual
201 selection on dimorphic traits also seems to vary geographically: most traits are under directional sexual
202 selection in multiple populations/subspecies, but the strength of selection varies geographically [38].
203 Here, we showed for the first time in any European barn swallow population that individual variation in
204 both the ‘visible’ and UV components of ventral plumage coloration predicts seasonal reproductive
205 success. Interestingly, in the same study population darker males suffer a viability disadvantage
206 compared to paler males [46], suggesting that conflicting fecundity and viability selection may concur
207 in maintaining extensive, genetically based polymorphism in coloration.

208 In conclusion, we showed for the first time in any bird species that telomere length covaries with both
209 reproductive success and coloration in both sexes. Because coloration reliably reflects TL, it may
210 mediate the observed positive assortative mating for TL, which may result from adaptive mutual choice

211 between high-quality males and females. Telomere length may therefore be an ultimate target of
212 (mutual) mate choice mediated by the expression of plumage colour traits, an hypothesis that can be
213 tested in several other bird species. Finally, our study highlights the importance of considering all the
214 different components of coloration of birds because these may have different role in social and sexual
215 communication.

216

217 **Authors' Contributions**

218 MP, AR, LC, NS conceived the study; AR, AC, DR, NS collected field data; MP, LK, MS, ZP, SGN,
219 EG performed laboratory analyses; AC performed color analyses; MP, AR, NS carried out the statistical
220 analyses; MP, AR, NS drafted the manuscript. All authors gave final approval for publication

221

222 **References**

- 223 1. Monaghan P, Haussmann MF. 2006 Do telomere dynamics link lifestyle and lifespan? *Trends Ecol.*
224 *Evol.* **21**, 47–53. (doi:10.1016/j.tree.2005.11.007)
- 225 2. Palm W, de Lange T. 2008 How shelterin protects mammalian telomeres. *Annu. Rev. Genet.* **42**,
226 301–34. (doi: 10.1146/annurev.genet.41.110306.130350)
- 227 3. Blackburn EH. 2000 Telomere states and cell fates. *Nature.* **408**, 53–56. (doi: 10.1038/35040500)4.
- 228 Campisi J, Kim S, Lim C, Rubio M. 2001 Cellular senescence, cancer and ageing: the telomere
229 connection. *Exp. Gerontol.* **36**, 1619–1637. (doi:10.1016/S0531-5565(01)00160-7)
- 230 5. Bize P, Criscuolo F, Metcalfe NB, Nasir L, Monaghan P. 2009 Telomere dynamics rather than age
231 predict life expectancy in the wild. *Proc. R. Soc. Lond. B.* **276**, 1679–1683. (doi:
232 10.1098/rspb.2008.1817)
- 233 6. Angelier F, Vleck CM, Holberton RL, Marra PP. 2013 Telomere length, non-breeding habitat and
234 return rate in male American redstart. *Funct. Ecol.* **27**, 342–350. (doi: 10.1111/1365-2435.12041)
- 235 7. Boonekamp JJ, Mulder GA, Salomons HM, Dijkstra C, Verhulst S. 2014 Nestling telomere
236 shortening, but not telomere length, reflects developmental stress and predicts survival in the wild.
237 *Proc. R. Soc. Lond. B.* **281**, 20133287. (doi: 10.1098/rspb.2013.3287)
- 238 8. Stier A, Viblanc VA, Massemin-Challet S, Handrich Y, Zahan S, Rojas ER, Saraux C, Le Vaillant
239 M, Prud'homme O, Grosbellet E, *et al.* 2014 Starting with a handicap: phenotypic differences
240 between early- and late-born king penguin chicks and their survival correlates. *Funct. Ecol.* **28**,
241 601–611. (doi: 10.1111/1365-2435.12204)
- 242 9. Caprioli M, Romano M, Romano A, Rubolini D, Motta R, Folini M, Saino N. 2013 Nestling
243 telomere length does not predict longevity, but covaries with adult body size in wild barn swallows.
244 *Biol. Lett.* **9**, 20130340. (doi: 10.1098/rsbl.2013.0340)

- 245 10. Pauliny A, Wagner RH, Augustin J, Szep T, Blomqvist D. 2006 Age independent telomere length
246 predicts fitness in two bird species. *Mol. Ecol.* **15**, 1681–1687. (doi: 10.1111/j.1365-
247 294X.2006.02862.x)
- 248 11. Le Vaillant M, Viblanc VA, Saraux C, Le Bohec C, Le Maho Y, Kato A, Criscuolo F, Ropert-
249 Coudert Y. 2015 Telomere length reflects individual quality in free-living adult king penguins.
250 *Polar Biol.* (doi 10.1007/s00300-015-1766-0)
- 251 12. Asghar M, Bensch S, Tarka M, Hansson B, Hasselquist D. 2015a Maternal and genetic factors
252 determine early life telomere length. *Proc. R. Soc. B.* **282**, 20142263. (doi:
253 10.1098/rspb.2014.2263)
- 254 13. Reichert S, Stier A, Zahn S, Arrivé M, Bize P, Massemin S, Criscuolo F. 2014 Increased brood
255 size leads to persistent eroded telomeres. *Front. Ecol. Evol.* **2**, 9. (doi: 10.3389/fevo.2014.00009)
- 256 14. Becker PJJ, Reichert S, Zahn S, Hegelbach J, Massemin S, Keller LF, Postma E, Criscuolo F.
257 2015 Mother–offspring and nest-mate resemblance but no heritability in early-life telomere length
258 in white-throated dippers. *Proc. R. Soc. B.* **282**, 20142924. (doi: 10.1098/rspb.2014.2924)
- 259 15. Hall ME, Nasir L, Daunt F, Gault EA, Croxall JP, Wanless S, Monaghan P. 2004 Telomere loss
260 in relation to age and early environment in long-lived birds. *Proc. R. Soc. Lond. B.* **271**, 1571–
261 1576. (doi: 10.1098/rspb.2004.2768)
- 262 16. Monaghan P. 2014 Organismal stress, telomeres and life histories. *J. Exp. Biol.* **217**, 57–66. (doi:
263 10.1242/jeb.090043)
- 264 17. Watson H, Bolton M, Monaghan P. 2015 Variation in early-life telomere dynamics in a long-
265 lived bird: links to environmental conditions and survival. *J. Exp. Biol.* **218**, 668–674. (doi:
266 10.1242/jeb.104265)

- 267 18. Asghar M, Hasselquist D, Hansson B, Zehtindjiev P, Westerdahl H, Bensch S. 2015b Hidden
268 costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds.
269 *Science*. **347**, 436–438. (doi: 10.1126/science.1261121)
- 270 19. Nettle D, Monaghan P, Gillespie R, Brilot B, Bedford T, Bateson M. 2015 An experimental
271 demonstration that early-life competitive disadvantage accelerates telomere loss. *Proc. R. Soc.*
272 *Lond. B*. **282**, 20141610. (doi: 10.1098/rspb.2014.1610)
- 273 20. Reichert S, Criscuolo F, Zahn S, Arrivé M, Bize P, Massemin S. 2015 Immediate and delayed
274 effects of growth conditions on ageing parameters in nestling zebra finches. *J. Exp. Biol.* **218**, 491–
275 499. (doi: 10.1242/jeb.109942)
- 276 21. Stier A, Massemin S, Zahn S, Tissier ML, Criscuolo F. 2015 Starting with a handicap: effects of
277 asynchronous hatching on growth rate, oxidative stress and telomere dynamics in free living great
278 tits. *Oecologia*. **179**, 999–1010. (doi: 10.1007/s00442-015-3429-9)
- 279 22. Costanzo A, Parolini M, Bazzi G, Korialuli L, Santagostino M, Possenti CD, Romano A,
280 Nergadze SG, Rubolini D, Giulotto E, *et al.* 2016 Brood size, telomere length, and parent-offspring
281 color signalling in barn swallows. *Behav. Ecol.* **28**, 204–211. (doi: 10.1093/beheco/arw147)
- 282 23. Bauch C, Becker PH, Verhulst S. 2013 Telomere length reflects phenotypic quality and costs of
283 reproduction in a long-lived seabird. *Proc. R. Soc. B*. **280**, 20122540. (doi:
284 10.1098/rspb.2012.2540)
- 285 24. Voillemot M, Hine K, Zahn S, Criscuolo F, Gustafsson L, Doligez B, Bize P. 2012 Effects of
286 brood size manipulation and common origin on phenotype and telomere length in nestling collared
287 flycatchers. *BMC Ecol.* **12**, 17. (doi: 10.1186/1472-6785-12-17)
- 288 25. Noguera JC, Metcalfe NB, Boner W, Monaghan P. 2015 Sex-dependent effects of nutrition on
289 telomere dynamics in zebra finches (*Taeniopygia guttata*). *Biol. Lett.* **11**, 20140938. (doi:
290 10.1098/rsbl.2014.0938)

- 291 26. Badás EP, Martínez J, Rivero de Aguilar Cachafeiro J, Miranda F, Figuerola J, Merino S. 2015
292 Ageing and reproduction: antioxidant supplementation alleviates telomere loss in wild birds. *J.*
293 *Evol. Biol.* **28**, 896–905. (doi: 10.1111/jeb.12615)
- 294 27. von Zglinicki T. 2002 Oxidative stress shortens telomeres. *Trends Biochem. Sci.* **27**, 339–344.
295 (doi: 10.1016/s0968-0004(02)02110-2)
- 296 28. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD. 2004 Accelerated telomere
297 shortening in response to life stress. *PNAS.* **101**, 17312–17315. (doi: 10.1073/pnas.0407162101)
- 298 29. Houben JMJ, Moonen HJJ, van Schooten FJ, Hageman GJ. 2008 Telomere length assessment:
299 bio-marker of chronic oxidative stress? *Free Radic. Biol. Med.* **44**, 235–246.
300 (doi:10.1016/j.freeradbiomed.2007.10.001)
- 301 30. Beaulieu M, Reichert S, Le Maho Y, Ancel A, Criscuolo F. 2011 Oxidative status and telomere
302 length in a long-lived bird facing a costly reproductive event. *Funct. Ecol.* **25**, 577–585. (doi:
303 10.1111/j.1365-2435.2010.01825.x)
- 304 31. Monaghan P. 2010 Telomere and life histories: the long and the short of it. *Ann. NY Acad. Sci.*
305 **1206**, 130–142. (doi: 10.1111/j.1749-6632.2010.05705.x)
- 306 32. Anderson M. 1994 *Sexual Selection*. Princeton: Princeton University Press.
- 307 33. Møller AP. 1994 *Sexual Selection and the Barn Swallow*. Oxford: Oxford University Press.
- 308 34. Turner A. 2006 *The Barn Swallow*. London: T & AD Poyser.
- 309 35. Saino N, Romano M, Rubolini D, Teplitski C, Ambrosini R, Caprioli M, Canova C, Wakamatsu
310 K. 2013a Sexual dimorphism in melanin pigmentation, feather coloration and its heritability in the
311 barn swallow (*Hirundo rustica*). *PLoS ONE.* **8**, e58024. (doi: 10.1371/journal.pone.0058024)
- 312 36. Romano A, Romano M, Caprioli M, Costanzo A, Parolini M, Rubolini D, Saino N. 2015 Sex
313 allocation according to multiple sexually dimorphic traits of both parents in the barn swallow
314 (*Hirundo rustica*). *J. Evol. Biol.* **28**, 1234–1247. (doi: 10.1111/jeb.12650)

- 315 37. Romano A, Bazzi G, Caprioli M, Corti M, Costanzo A, Rubolini D, Saino N. 2016a Nestling sex
316 and plumage color predict food allocation by barn swallow parents. *Behav Ecol* **27**, 1198–1205.
317 (doi:10.1093/beheco/arw040)
- 318 38. Romano A, Costanzo A, Rubolini D, Saino N, Møller AP. 2016b Geographical and seasonal
319 variation in the intensity of sexual selection in the barn swallow *Hirundo rustica*: a meta-analysis.
320 *Biol. Rev.* (doi: 10.1111/brv.12297)
- 321 39. Saino N, Romano M, Ambrosini R, Rubolini D, Boncoraglio G, Caprioli M, Romano A. 2012
322 Longevity and lifetime reproductive success of barn swallow offspring are predicted by their
323 hatching date and phenotypic quality. *J. Anim. Ecol.* **81**, 1004–1012. (doi: 10.1111/j.1365-
324 2656.2012.01989.x)
- 325 40. Parolini M, Romano A, Khorauli L, Nergadze SG, Caprioli M, Rubolini D, Santagostino M,
326 Saino N, Giulotto E. 2015 Early-life telomere dynamics differ between the sexes and predict
327 growth in the barn swallow (*Hirundo rustica*). *PLoS ONE.* **10**, e0142530.
328 (doi:10.1371/journal.pone.0142530)
- 329 41. Stoddard MS, Prum RO. 2008 Evolution of avian plumage color in a tetrahedral color space: a
330 phylogenetic analysis of new world buntings. *Am. Nat.* **171**, 755–776. (doi: 10.1086/587526)
- 331 42. Reichert S, Bize P, Arrivé M, Zahn S, Massemin S, Criscuolo F. 2014b. Experimental increase in
332 telomere length leads to faster feather regeneration. *Exp. Gerontol.* **52**, 36–38.
333 (<http://dx.doi.org/10.1016/j.exger.2014.01.019>)
- 334 43. Chan SRWL, Blackburn EH. 2004 Telomeres and telomerase. *Phil. Trans. R. Soc. Lond. B.* **359**,
335 109–121. (doi: 10.1098/rstb.2003.1370)
- 336 44. Bagheri S, Nosrati M, Li S, Fong S, Torabian S, Rangel J, Moore DH, Federman S, LaPosa RR,
337 Baehner FL, *et al.* 2006 Genes and pathways downstream of telomerase in melanoma metastasis.
338 *Proc. Natl. Acad. Sci. USA.* **103**, 11306–11311. (doi: 10.1073/pnas.0510085103)

- 339 45. Ducrest AL, Keller L, Roulin A. 2008 Pleiotropy in the melanocortin system, coloration and
340 behavioural syndromes. *Trends Ecol. Evol.* **23**, 502–510. (doi:10.1016/j.tree.2008.06.001)
- 341 46. Saino N, Romano M, Rubolini D, Ambrosini R, Caprioli M, Milzani A, Costanzo A, Colombo G,
342 Canova L, Wakamatsu K. 2013b Viability is associated with melanin-based coloration in the barn
343 swallow (*Hirundo rustica*). *PLoS One.* **8**, e60426. (doi: 10.1371/journal.pone.0060426)

344

345 Table 1. Poisson generalized linear models of seasonal reproductive success (total number of offspring
346 in the breeding season) in relation to RTL and age for females and males separately. Estimated
347 coefficients (SE) are given. Sample size was 65 females and 65 males.

	χ^2_1	<i>P</i>	coefficients (SE)
351 Females			
352 RTL	5.61	0.018	0.654 (0.276)
353 Age	7.09	0.008	0.254 (0.100)
354 Males			
355 RTL	4.60	0.032	0.568 (0.265)
356 Age	3.45	0.063	0.182 (0.098)

358

359 Table 2. Gaussian linear mixed models of ventral plumage tetrahedral colour components in relation to
 360 sex, RTL and age. The effects of age and of the RTL by sex interaction were removed when non-
 361 significant. The random effect of breeding pair was always included in the model. Estimated marginal
 362 means (EMM) and coefficients (SE) are given. Sample size was 65 males and 62 females for all
 363 analyses.

	<i>F</i>	df	<i>P</i>	EMM (SE) /coefficients (SE)	
367 θ					
368 Sex	19.31	1,123	< 0.001	males: 0.217 (0.008)	females: 0.266 (0.008)
369 RTL	2.74	1,123	0.100		
370 Sex \times RTL	3.97	1,123	0.048	males: -0.109 (0.041)**	females: 0.009 (0.041)
371 Age ^a	2.97	1,122	0.087		
372 φ					
373 Sex	13.70	1,124	< 0.001	males: -0.868 (0.008)	females: -0.912 (0.008)
374 RTL	6.07	1,124	0.015	0.072 (0.029)	
375 Sex \times RTL ^a	0.00	1,122	0.976		
376 Age ^a	0.73	1,122	0.395		
377 rA					
378 Sex	13.33	1,123	< 0.001	males: 0.211 (0.006)	females: 0.179 (0.006)
379 RTL	0.19	1,123	0.661		
380 Sex \times RTL	6.46	1,123	0.012	males: 0.046 (0.029)	females: -0.065 (0.032)*
381 Age ^a	0.01	1,122	0.906		

383 ^a: term removed from final model; *: 0.01 < *P* < 0.05; **: *P* < 0.01.

384

385 Table 3. Poisson generalized linear models of seasonal reproductive success (number of offspring in
 386 the breeding season) in relation to ventral plumage tetrahedral colour components and age for females
 387 and males separately. Estimated coefficients (SE) are given. Sample size was 62 females and 65 males.

	Females			Males			
	χ^2_1	<i>P</i>	coefficient (SE)	χ^2_1	<i>P</i>	coefficient (SE)	
392							
393	θ	1.90	0.169	1.190 (0.864)	5.48	0.019	-2.168 (0.926)
394	Age	6.55	0.011	0.252 (0.099)	2.40	0.122	0.155 (0.100)
395							
396	φ	0.00	0.958	-0.044 (0.835)	5.39	0.020	1.924 (0.828)
397	Age	5.56	0.018	0.228 (0.097)	2.65	0.103	0.161 (0.099)
398							
399	rA	1.82	0.177	-1.521 (1.127)	0.25	0.620	0.574 (1.158)
400	Age	5.91	0.015	0.235 (0.097)	3.53	0.060	0.184 (0.098)

402

403 **Legend to figures**

404

405 Figure 1. Seasonal reproductive success (total number of nestlings in the breeding season) in relation
406 to relative telomere length (RTL) of male or female barn swallows. The relationship was significantly
407 positive in both sexes.

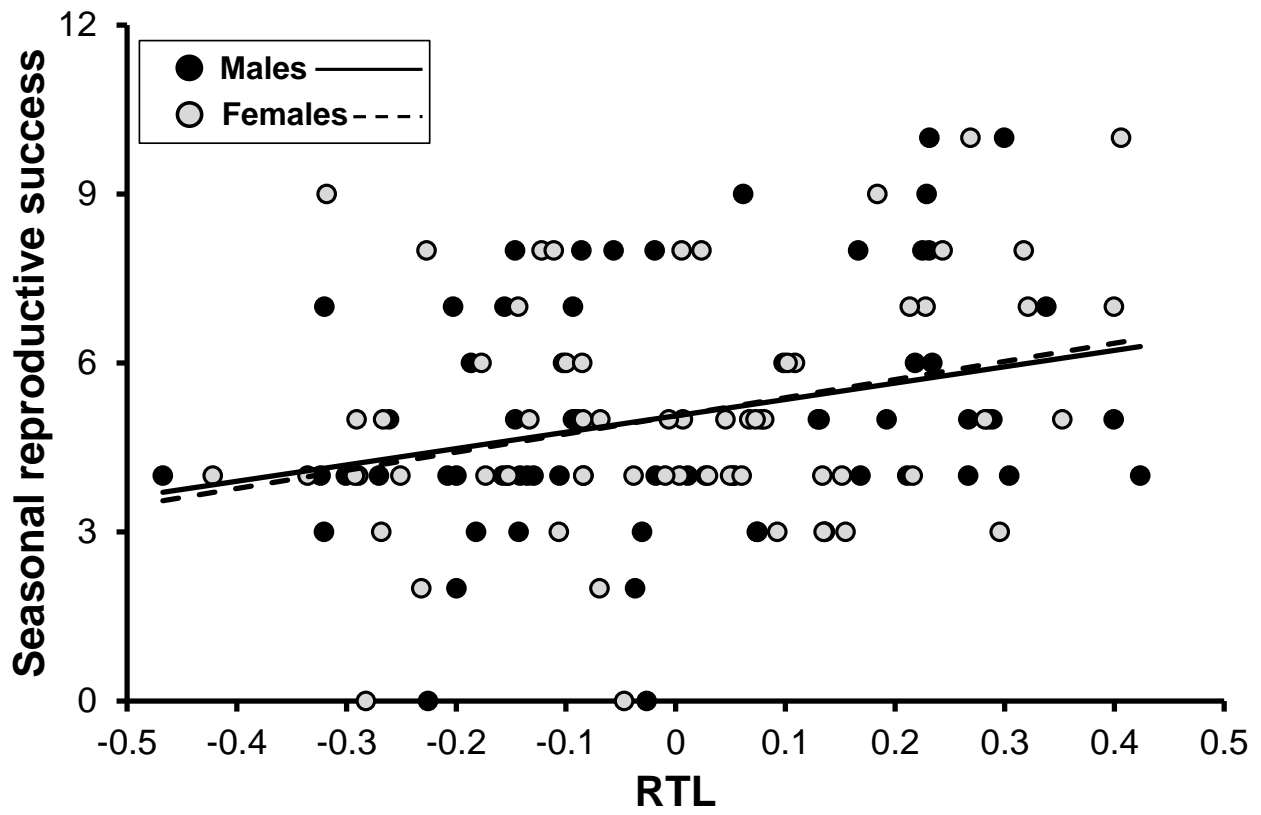
408

409 Figure 2. Tetrahedral components (θ , ϕ , rA) of the ventral plumage coloration of male and female
410 adult barn swallows in relation to relative telomere length (RTL). Linear regression lines for either sex
411 are shown. θ values significantly increased with RTL in males but not in females. ϕ values
412 significantly increase with RTL in both sexes. rA significantly declined with RTL in females but did
413 not significantly covary with RTL in males.

414

415 **Figure 1**

416



417

418

