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## ► To cite this version:

Steve Smith, Franz Hoelzl, Sandrine Zahn, François Criscuolo. Telomerase activity in ecological studies: What are its consequences for individual physiology and is there evidence for effects and trade-offs in wild populations. *Molecular Ecology*, 2021, 10.1111/mec.16233 . hal-03608073

**HAL Id: hal-03608073**

**<https://hal.science/hal-03608073v1>**

Submitted on 14 Mar 2022

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1 **Telomerase activity in ecological studies: what are its consequences for individual physiology and**  
2 **is there evidence for effects and trade-offs in wild populations**

3

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22 Running title: Telomerase and fitness

23

24 **Word count:**

25

26 Key words: Telomeres, trade-off, immunity, reproductive effort, life history

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34 **Abstract**

35 Increasing evidence at the cell level highlights the balance between investment in growth,  
36 reproduction and somatic maintenance in wild populations. Studies of telomere dynamics have  
37 informed researchers about the loss and gain of telomere length both on a seasonal scale and across  
38 the lifespan of individuals. In addition, telomere length and telomere rate of loss seems to have  
39 evolved differently among taxonomic groups, and relate differently to organismal diversity of lifespan.  
40 So far, the mechanisms behind telomere maintenance remain elusive, although many studies have  
41 inferred a role for telomerase, an enzyme/RNA complex known to induce telomere elongation from  
42 lab studies. Exciting further work is also emerging that suggests telomerase (and/or its individual  
43 component parts) has a role in fitness that goes beyond the maintenance of telomere length. Here we  
44 review the literature on telomerase biology and examine the evidence from ecological studies for the  
45 timing and extent of telomerase activation in relation to life history events associated with telomere  
46 maintenance. We suggest that the underlying mechanism is more complicated than originally  
47 anticipated, possibly involves several complimentary pathways, and is likely associated with high  
48 energetic costs. Potential pathways for future research are numerous and we outline what we see as  
49 the most promising prospects to expand our understanding of individual differences in immunity or  
50 reproduction efficiency.

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## 53 **Introduction**

54 The burgeoning field of telomere dynamics has added new perspectives and directions to the study of  
55 ecology and evolutionary biology. The interest is testament to the fact that telomeres represent an  
56 intriguing biomarker that provides the link between individual physiology, life history, and fitness. As  
57 the field has developed, so too has the realisation that the maintenance of telomeres is at the centre  
58 of this balance. Studies so far, both experimental (Herborn et al., 2014; Nettle et al., 2015) and  
59 correlational (Hoelzl, Cornils, Smith, Moodley, & Ruf, 2016a; Turbill, Smith, Deimel, & Ruf, 2012),  
60 indicate that telomere restoration is costly (reviewed in Young, 2018). However, the mechanisms  
61 underlying telomere maintenance remain elusive and evolutionary biologists have turned to clinical  
62 research for answers. The obvious candidate as regulator within the system is the enzyme complex  
63 telomerase. Telomerase comprises an RNA component (*TERC*) and a catalytic protein domain (*TERT*)  
64 essential for the reverse transcriptase activity of the enzyme (see Box 1). Telomerase is known to  
65 catalyse the elongation and maintenance of telomeres by adding telomeric repeats to the  
66 chromosomal ends and thus counteracting losses that occur during end replication and oxidative stress  
67 (Harley, Futcher, & Greider, 1990; von Zglinicki, 2002). For most organisms, telomerase is not active  
68 throughout all life stages and across all tissue types (Hausmann, Winkler, Huntington, Nisbet, & Vleck,  
69 2004; Lingner et al., 1997; Wright, Piatyszek, Rainey, Byrd, & Shay, 1996), strongly suggesting trade-  
70 offs between life history stages such as growth, maintenance and reproduction. As a potential  
71 regulator of these trade-offs, measurement telomerase is of increasing interest for evolutionary  
72 biologists trying to understand the process of senescence (see Box 2).

**Box 1****The structure and function of telomerase**

-Telomerase in its active state is composed of a DNA polymerase enzyme complex (telomerase reverse transcriptase; TERT) that carries its own RNA template (telomerase RNA component; *TERC*, TER or TR) to add telomeric repeats to the ends of chromosomes.

-In vertebrates, TERT is composed of the essential telomerase N-terminal domain (TEN), the C-terminal extension domain (CTE), the telomerase RNA-binding domain (TRBD) and the reverse transcriptase domain (RT). The TEN domain stabilizes the DNA-RNA duplex in the active site of the enzyme (Akiyama, Parks, & Stone, 2015), CTE is necessary for stable binding of the enzyme to the DNA (Hossain, Singh, & Lue, 2002), TRBD binds the RNA-template (Lai et al, 2001) and RT is the catalytic domain (Dey & Chakrabarti, 2018).

-*TERC* is considered a non-coding RNA that contains the template region for adding telomeric repeats to the ends of chromosomes. Its total size is highly variable between taxa and ranges from 133 nucleotides (nt) in the fish *Nothobranchius furzeri* to over 2000 nt in the fungus *Neurospora crassa* (<http://telomerase.asu.edu/>; Podlevsky, Bley, Omana, Qi, & Chen, 2008). In vertebrates, the template-RNA is flanked by the template boundary element (TBE) at the 5' side. The pseudoknot (PK) is located downstream of the template and is essential for TERT-*TERC* interaction and function of the holoenzyme. Furthermore, *TERC* contains several structures that interact with TERT and other proteins (e.g. dyskerin protein complex) to ensure functionality (reviewed in Logeswaran, Li, Podlevsky, & Chen, 2021); <http://telomerase.asu.edu/>).

-Telomeric repeat variants (e.g. TTAGGG - in vertebrates) differ between taxonomic groups. Correspondingly, both TERT and *TERC* have a distinct composition of subunits and regions in different taxa. In insects for example, TERT lacks the TEN domain while nematodes lack TEN and large fractions of CTE (reviewed in Mason, Schuller, & Skordalakes, 2011). In *TERC*, the arrangement and composition of elements (PK, TBE, ect.) is variable between taxa, but even more pronounced, the sequence and structure within these elements is highly variable between taxonomic groups (Logeswaran et al., 2021).

-Trafficking of TERT and *TERC* between nucleus and cytoplasm is not fully understood. In yeast (*Saccharomyces cervicia*), the assembly of the telomerase holoenzyme likely takes place in the cytoplasm before it is imported into the nucleus, while in humans, TERT and *TERC* are separated in the nucleus during most of the cell cycle and are only assembled during S phase. Such separation is likely to be necessary to avoid unregulated telomerase activity (reviewed in Gallardo & Chartrand, 2008). Telomerase activity is detectable throughout the cell cycle but only a small fraction of *TERC* is co-localized with telomeres during S phase (the stage where telomere elongation takes place *in vivo*), suggesting a low elongation rate per cell cycle (this is true for both, human and yeast; Gallardo & Chartrand, 2008; Teixeira, Arneric, Sperisen, & Lingner, 2004).

-A study by Schmidt, Zaug, and Cech (2016) reveals that telomerase diffuses throughout the nucleoplasm and that the enzyme frequently binds TPP1 (a protein of the shelterin complex) at telomeres. These bindings occur thousands of times per S phase, but only a very limited number of these probing events are sufficiently long to allow telomere elongation.

-After telomerase is guided to the chromosomal end, and forms stable "bonds", elongation is carried out only on the G-rich overhang (either resulting from terminal RNA primer removal on the lagging strand, or by exonuclease dependent resection, reviewed in Procházková Schruppová and Fajkus (2020). Therefore, telomerase binds to the distal nucleotides of these overhangs and adds a new telomeric repeat (in vertebrates: GGTTAG). This elongation step can be repeated multiple times via a translocation mechanism and DNA Pol [alpha]-primase can fill the complementary strand (Diede & Gottschling, 1999).

-In multicellular organisms, telomerase activity is usually detected in all early developmental stages. After organogenesis, telomerase activity is restricted to proliferating stem cells, while it is downregulated in somatic cells (reviewed in Procházková Schruppová, Fojtová, & Fajkus, 2019). In mammals, the tendency to downregulate somatic telomerase activity is body mass dependent, where species of weight smaller than 1 kg (e.g. mice) keep telomerase activity throughout their life, while larger animals downregulate telomerase in somatic tissues (likely to reduce the risk of cancer; Seluanov et al., 2007).

74 Senescence is defined in evolutionary biology as the relaxation of natural selection at old-ages,  
75 attributed to the reduced fitness contribution of older breeders due to poor reproductive  
76 performances and survival rates (Gaillard & Lemaître, 2020). Williams' theory of senescence stipulates  
77 that it should be found in all living organisms that have, among other prerequisites (Gaillard &  
78 Lemaître, 2017), genes that will be beneficial early in life and detrimental later on (i.e. (the antagonistic  
79 pleiotropy of aging, Williams, 1957). At a more mechanistic level, senescence, which promotes cell  
80 division arrest and apoptosis, could be considered as a cell response to aging stress, with both positive  
81 and negative impacts on individual fitness. For instance, cell senescence also promotes regular tissue  
82 repair, and protects against cell immortalization and cancer, while at the same time triggering pro-  
83 inflammatory or tissue degeneration processes (reviewed in Autexier & Lue, 2006; Campisi, 2013).  
84 Because telomeres and telomerase stand at the crossroad of these cell mechanisms, we propose here  
85 that the study of telomerase will benefit from being considered in the context of its pleiotropic effects  
86 on individual fitness and through its modulation of life-history trade-offs. To do so, we highlight that  
87 **(i)** telomerase genes vary in sequences and expression, **(ii)** telomerase may act via non-telomere-  
88 related pathways, **(iii)** telomerase may have pleiotropic effects due to dynamic changes in expression  
89 and thus mediate trade-offs among traits. We then address what could be the costs associated to  
90 telomerase activation, and propose a few avenues for studying the putative telomerase – fitness link.

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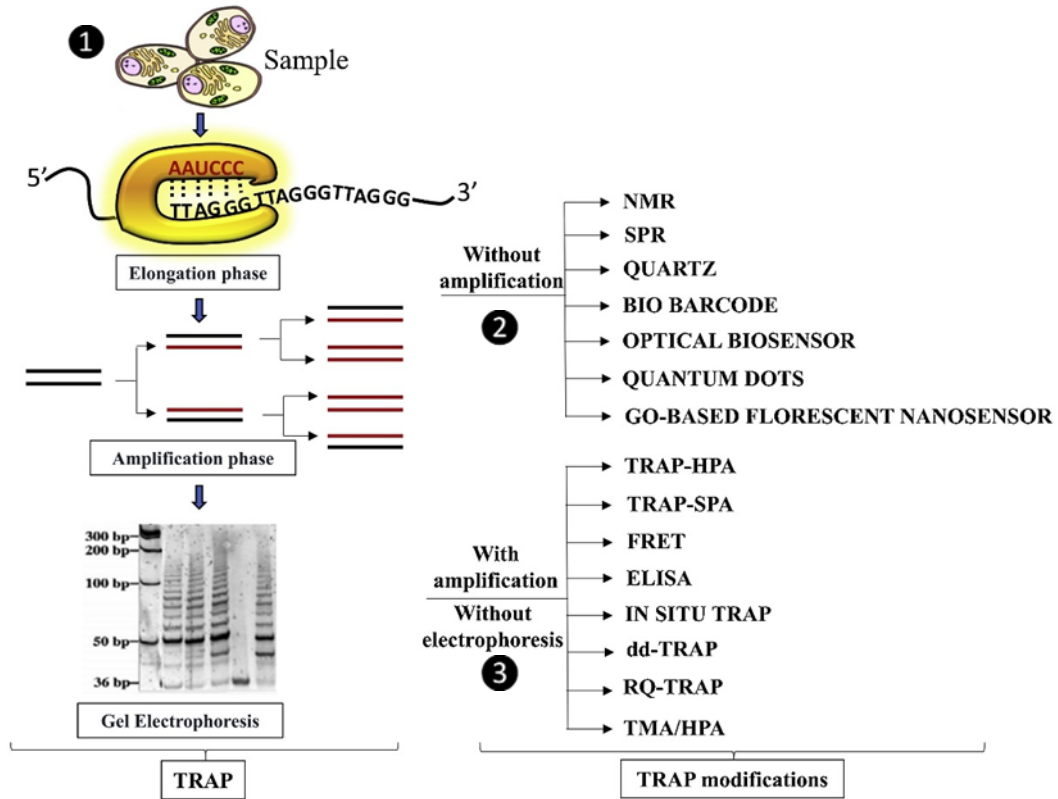
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**Box 2: Measurement of telomerase activity: previous studies and methods employed**

**Techniques employed to quantify telomerase activity (TA)**



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101 For more than a decade, evolutionary ecologists have focussed on telomeres (Hausmann & Vleck,  
 102 2002), and very rapidly to telomerase activity (Hausmann, Winkler, Huntington, Nisbet, & Vleck,  
 103 2007), in order to better understand individual variability in lifespan (Hausmann, Winkler, & Vleck,  
 104 2005). However, evaluations of telomerase activity are not a simple prospect for most of the species  
 105 of ecological interest. Logistical constraints and/or specific expertise requirements are the major  
 106 challenges. For instance, storage and pre-treatment of samples to preserve telomerase activity is  
 107 inherently complicated ①. Thus the development of reliable telomerase activity (TA) assessment  
 108 methodologies is essential to allow research in evolutionary biology to develop and understand co-  
 109 variation of age- or tissue-related telomerase activity with animal life-histories. This schematic  
 110 representation shows the most common methods employed to measure TA in human and cancer  
 111 research. The methods are presented following two main categories: ② detection of telomerase  
 112 products and ③ amplification of these products (TRAP-based methods). Figure is adapted from Mensà  
 113 et al. (2019).

114

115 **The Ecological Link to Telomerase**

116 Early studies of telomere dynamics in non-model organisms reported individual differences in  
 117 telomere attrition related to age (Aubert, 2014; 2020), birth order (Noguera, Metcalfe, Reichert, &  
 118 Monaghan, 2016), birth timing (Eisenberg, Hayes, & Kuzawa, 2012), early life stress (Cram, Monaghan,

119 Gillespie, & Clutton-Brock, 2017; Price, Kao, Burgers, Carpenter, & Tyrka, 2013), reproductive output  
120 (Beaulieu, Reichert, Le Maho, Ancel, & Criscuolo, 2011; Monaghan & Hausmann, 2006), rewarming  
121 during hibernation (Hoelzl et al., 2016b), and habitat suitability (Angelier, Vleck, Holberton, & Marra,  
122 2013) among others. After this initial interest in telomeres as a reliable biomarker for stress and  
123 biological aging, evidence began to emerge that, in some species at least, telomere length could be  
124 actively restored or even elongated (Foley et al., 2018; Hausmann et al., 2003; Hoelzl et al., 2016b).  
125 Despite initial scepticism, further studies confirmed active telomere maintenance across multiple  
126 species. Telomerase activity was considered the most likely mechanism responsible for telomeric  
127 restoration and once again ecological researchers borrowed from clinical fields and cancer research to  
128 find the best way to measure telomerase activity in an ecological setting (see Box 2). Subsequent work  
129 has implicated an association between telomerase activity levels and telomere dynamics in a broad  
130 range of taxa. Mu et al. (2015) found a link between seasonal leaf growth, telomere length and  
131 telomerase activity in the Chinese Pine (*Pinus tabulaeformis*). Interestingly, telomere length also  
132 increased with temperature throughout the year whereas telomerase activity showed a negative  
133 association, suggesting a potential lag between stimulus and response. A strong association between  
134 telomerase activity and telomere length was observed in multiple strains of zebra fish with a marked  
135 drop in both detected in older individuals (Anchelin, Murcia, Alcaraz-Pérez, García-Navarro, & Cayuela,  
136 2011). In a follow-up experimental study (Anchelin et al., 2013), telomerase-knockdown zebra fish  
137 showed premature aging and reduced lifespan in the first generation. Restoration of telomerase  
138 activity in the second generation led to rescue of telomere length and survival confirming the causal  
139 role of telomerase in the association. Likewise, Hatakeyama et al. (2016) demonstrated in Japanese  
140 medaka (*Oryzias latipes*) that telomere attrition and restoration are linked to growth and telomerase  
141 activity respectively, and that a critical loss of telomere homeostasis is associated with mortality.  
142 Recently, an intriguing link with embryo corticosterone levels was shown in yellow-legged gulls  
143 (Noguera et al. 2020). Hatchlings from corticosterone-injected eggs were shown to have both higher  
144 levels of telomerase activity and longer telomeres. Telomerase was also shown to induce the synthesis



145 and repair of TTAGG-telomeric repeats in Lepidoptera (Gong et al. 2015) and this association seems to  
146 be widespread in most orders of insects (Korandova et al. 2014). Combined, these studies largely  
147 demonstrate that telomere length and telomerase is closely coupled as expected based on *in vitro*  
148 studies and tightly regulated by environmental factors.

149 Crucially though, many studies have not detected the expected link between telomere maintenance  
150 and telomerase activity (see citations in Supp. Table 1). Clinical studies in humans and experimental  
151 work show that in many conditions, telomere length is decoupled from telomerase activity (Xie et al.,  
152 2015) and that telomerase acts in diverse functions at cellular and physiological levels (de Punder,  
153 Heim, Wadhwa, & Entringer, 2019; Haendeler et al., 2009; Sahin & DePinho, 2012; Saretzki, 2009).  
154 Work in honeybees (*Apis mellifera*) also could not find a link between telomere length and telomerase  
155 activity, which was found to be regulated in a developmental and caste-specific manner (Korandová &  
156 Čapková Frydrychová, 2015; see also Koubová et al., 2019). The inconsistent findings from studies of  
157 the telomere – telomerase relationship raise questions about the ubiquity of a single telomere repair  
158 mechanism but, perhaps more interestingly, also suggests alternative roles for telomerase and its  
159 subunits in life history trade-offs. Resolving the links in these trade-offs promises to open the door to  
160 exciting avenues of research in ecology and evolutionary biology.

### 161 **Linking life-history trade-offs and individual fitness with telomerase?**

162 The essential requirement for a biological variable to play a role in evolution is to show  
163 variation among individuals in a population (along with transmission to the next generation and  
164 conferring fitness effect). Telomerase is formed *via* the assemblage of two main subunits coded by  
165 highly conserved genes (*TERT*, *TERC*), which are also characterized by a recent evolution history  
166 (Hrdličková, Nehyba, Lim, Grützner, & Bose Jr., 2012). More importantly, polymorphisms of those  
167 genes do exist in humans, their discovery originating from the study of telomere diseases such as  
168 congenital dyskeratosis (Savage et al., 2008; Vulliamy et al., 2001). In those patients, the RNA  
169 component of the telomerase (*TERC*) and an associated regulating protein (dyskerin, *DCK1* gene) are

170 mutated, leading to defective telomere maintenance by telomerase and to premature ageing  
171 phenotypes (Marrone & Dokal, 2004). Human *TERT* mutations were also found to be associated with  
172 different, rather late-life, diseases (Calado & Young, 2012), and *TERT* single nucleotide polymorphisms  
173 (SNPs) have been identified in humans, often associated with an increase in cancer risks (Rafnar et al.,  
174 2009, together with shortened telomeres in this study), or bone marrow failure (Yamaguchi et al.,  
175 2005). Enhanced telomerase activity is found in more than 9 out of 10 cancer cells (Shay & Bacchetti,  
176 1997). So how could telomerase defects enhance cancer risks? Causality is thought to relate to the  
177 capacity of hematopoietic cells (and then immune cells) to up-regulate telomerase when activated.  
178 Given that the level of *TERT* expression may be the rate-limiting mechanisms of control of telomerase  
179 activity (Counter et al., 1998), *TERT* deleterious mutations may shorten telomere length of leucocytes,  
180 and then decrease the ability of the immune system to survey cell immortalization and eliminate  
181 cancer cells (Calado & Young, 2012). Therefore, focusing on the individual variation in *TERT* expression  
182 and telomerase activity in white-blood cells, and characterising how it may modulate  
183 immunosenescence (de Punder et al., 2019), may provide the first insights into the telomerase – fitness  
184 relationship. In addition, telomerase polymorphisms have been previously associated with non-cancer  
185 diseases, with significantly modified soma protective effects (Snetselaar, van Oosterhout, Grutters, &  
186 van Moorsel, 2018). *TERC* variants were shown to have different longevities in humans (Soerensen et  
187 al., 2012), thereby suggesting that some telomerase mutations may carry putative fitness benefits.

188 *Telomerase: a two-headed coin with telomere length and non-telomere length sides*

189 The studies cited above mainly explain their results based on the protective action of  
190 telomerase on telomeres, which is of prime interest for biologists seeking for telomerase – fitness  
191 relationships but also renders difficult any conclusion on telomerase effect *per se*. However, non-  
192 canonical effects of telomerase do exist (*i.e.* not mediated by the maintenance of telomere length) and  
193 should be taken into account to explore further the potential fitness benefits provided by this enzyme.  
194 Insights for the putative importance of these non-canonical roles in shaping life-histories were recently

195 illustrated in an interspecific comparison study of *TERT* sequences and expressions (Lai et al., 2017).  
196 Highly variable patterns of alternative splicing of the catalytic subunit *TERT* was described even in  
197 closely related species, suggesting that different functions of telomerase may have evolved in relation  
198 to lineage specific life histories (Lai et al., 2017). However, to date, the nature of those non-canonical  
199 roles (and their control by alternative splicing) are almost exclusively studied in humans and seem  
200 restricted to a few main impacts of *TERT* expression: mitochondrial functioning, enhanced cell  
201 proliferation (*via* the activation of growth factor signalisation pathway), control of the gene expression  
202 (not only oncogenes, *e.g.* immune genes) and regulation of telomerase activity during early-life  
203 development (de Punder et al., 2019; Smith, Coller, & Roberts, 2003; Ulaner, Hu, Vu, Giudice, &  
204 Hoffman, 1998; Xiang, Wang, Mao, & Li, 2000). Even if we need additional aspects of telomerase  
205 activity or *TERT* to be better described (like non-telomeric DNA maintenance, Gorbunova, Seluanov, &  
206 Pereira-Smith, 2002), those observations are of broad interest for evolutionary biologists. They  
207 suggests that either due to genetic variance or post-transcriptional regulation, individuals may  
208 differently react to environmental factors, for which the physiological response needs the activation  
209 of *TERT* expression and/or telomerase activity. One intriguing observation was made in planarian  
210 worms, an animal showing tissue regeneration capacity and asexual or sexual reproduction. In this  
211 model, alternative splicing of *TERT* seems to regulate the mechanisms of telomere elongation either  
212 in the somatic cells during fission (asexual reproduction) or in the germ-line cells during sexual  
213 reproduction (Tan et al., 2012). Together with similar observations in colonial ascidians or urochordate  
214 animals, it suggests that variation in telomerase expression has coevolved with specific life-history  
215 traits like mode of reproduction (Lai et al., 2017), extreme lifespan or somatic regeneration capacity  
216 (Tan et al., 2012 and herein cited references).

### 217 *Tackling the telomerase – fitness link from its pleiotropic nature*

218 We have briefly introduced so far that the role of telomerase may go beyond its accepted  
219 contribution to the maintenance of telomere length (non-canonical impacts of the *TERT* or *TERC*

220 subunits), which may be associated with species' traits. However, to tackle the telomerase – fitness  
221 hypothesis, we may also reconsider the question through the prism of its main period of high activity  
222 (at least in most birds and mammals), early-life development, and of its primary role in cell replication.  
223 Could telomerase have a dual and age-related impact on fitness, *via* individual health and lifespan  
224 components? By favouring developmental processes and cell renewal in early life, do telomerase  
225 activity and non-canonical *TERT/TERC* expression have positive effects on individual reproductive  
226 output, while leading to increased cancer risks in later life? When thinking of such pleiotropies of  
227 telomerase and fitness relationships, the first objective of future studies should be to evaluate how  
228 early and late-life expression of telomerase may modulate individual fitness at young, reproductive  
229 and post-reproductive adult stages. For instance, *TERT* alternative splicing is recognized as being  
230 involved in modulating telomerase activity during development in humans, but only its consequence  
231 in terms of telomere length has so far been evaluated (reviewed in Shay & Wright, 2019). This question  
232 needs to be re-evaluated in biological models where individual health and fitness can be measured  
233 over life. Along the same lines, the pleiotropies of non-canonical effects should also be studied. In fact,  
234 *TERT* ectopic expression in mice fibroblasts triggers cell growth by repressing growth-inhibiting  
235 pathways (Geserick, Tejera, González-Suárez, Klatt, & Blasco, 2006). This suggests that individual  
236 variation in *TERT* expression may underline individual variation in growth rates, and thereby contribute  
237 to the growth – lifespan trade-off (Metcalf & Monaghan, 2003).

238 *Tackling the telomerase – fitness link from its dynamic nature*

239 An important second way of studying telomerase in an evolutionary context will be to search  
240 for acute telomerase activity or *TERT* expression in response to stress under natural conditions.  
241 Telomerase is mainly described as active during embryogenesis and early-life in most animals (Bekaert,  
242 Derradji, & Baatout, 2004; Prowse & Greider, 1995), and then is shutdown likely to avoid cancer-risks  
243 (Shay & Wright, 2019). However, this general view has been regularly amended by data collected on  
244 several taxa, suggesting that telomerase activity could be maintained at adulthood in certain

245 conditions. For instance, beside the maintained telomerase activity in the soma of insects, lizards or  
246 fish (reviewed in Gomes, Shay, & Wright, 2010), repression of telomerase activity was not found as an  
247 unalterable characteristic in mammals and birds. Telomerase activity actually covaries (negatively)  
248 with species body mass in rodents (Gorbunova & Seluanov, 2009) and life-long telomerase activity  
249 characterizes some long-lived small seabirds (Hausmann et al., 2007), while its expected positive  
250 impact on telomere length was described in hibernating (Hoelzl et al., 2016a; Turbill, Ruf, Smith, &  
251 Bieber, 2013) as well as in non-hibernating species (Criscuolo, Pillay, Zahn, & Schradin, 2020; Fairlie et  
252 al., 2016). The latter studies highlighted that telomerase may be turned-on at certain life stages, likely  
253 in some particular cells (*e.g.* stem or hematopoietic cells) and raises the question of whether  
254 telomerase forms part of the response-repertoire an organism possesses to face stress (Beery et al.,  
255 2012), or which may constrain adaptive abilities (Choi, Fauce, & Effros, 2008). Given the ability of  
256 immune cells to express high telomerase activity when activated (Bodnar, Kim, Effros, & Chiu, 1996;  
257 Hiyama et al., 1995; Yamada, Motoji, & Mizoguchi, 1996), future studies would benefit from a focus  
258 on immune cells, and how changes in telomerase activity (*i.e.* increases) vary at the individual level in  
259 response to immune challenge and what are the collateral effects on other somatic tissues. Still, while  
260 exploratory, those studies will probably uncover only part of the global picture as they will focus solely  
261 on the dynamics of telomerase activity. Adding the non-canonical functions of telomerase *via* the  
262 characterization of *TERT* / *TERC* dynamics of expression over age and in response to stress is vitally  
263 important to establish how telomerase may affect individual fitness or bestow individuals with specific  
264 life-history trait associations.

### 265 *Telomerase as a mediator of the reproductive trade-off*

266 The different age-related risks of telomerase activity have so far been conceptualized based  
267 on our present knowledge of telomerase implication in cell-growth or renewal processes (Morrison,  
268 Prowse, Ho, & Weissman, 1996) and in cell immortalization and the associated cancer risk (Tian et al.,  
269 2018). However, the risk has never been considered in relation to the cost of reproduction in adults,

270 neither for telomerase activity nor at the level of subunit expression and of their non-canonical effects.  
271 When studying the cost of reproduction, defined as decreased future reproductive and survival rates  
272 (Stearns, 1992), one may consider telomerase as one of the pleiotropic regulators that may affect adult  
273 breeders current success (*via* the maintenance of telomere length) and their future prospects (*via* the  
274 non-canonical *TERT* / *TERC* protective effects, see below). Because the cost of reproduction may be  
275 mediated through telomere erosion or other cell defects of the adult soma and of gametes, using  
276 telomerase as the response variable will extend our understanding of reproductive costs beyond the  
277 “Y model” of energy allocation between reproduction and lifespan (reviewed in Harshman & Zera,  
278 2007). Such a possibility has been illustrated in a single cell-organism, yeast, where a role for  
279 telomerase in response to DNA replication stress, but also in additional maternal cell ageing, was  
280 highlighted (Xie et al., 2015). Such a telomerase-based fitness component may also relate to the  
281 regulation by telomerase of stem cells capacity to be mobilized and punctually proliferate (Calado &  
282 Chen, 2006), thereby modulating cell renewal rate and organ performance in breeders. Again, focusing  
283 on the immune system, as it is the somatic tissue where telomerase activity is more likely to be  
284 recorded, seems the best alternative if we are to evaluate how telomerase may mediate the ageing  
285 outcomes of reproduction (Schulenburg, Kurtz, Moret, & Siva-Jothy, 2009).

286

## 287 **Evidences for a cost of telomerase activation**

288         The first cost to consider for telomerase activity in an evolutionary biology sense is energetic.  
289 A previous review on the role of telomeres in the evolution of life-history traits underlined the  
290 importance (and lack) of obtaining accurate data on how costly it is for a cell, and thereby for an  
291 organism, to maintain telomere length (and thus indirectly in activating telomerase, Young, 2018).  
292 Ecophysiological studies have suggested that maintenance of telomere length may be interpreted as  
293 energetically costly since, for instance, food supply during bad years allowed better telomere  
294 maintenance in free-living edible dormice (*Glis glis*), (Hoelzl et al., 2016a; Nowack et al., 2019). Still,

295 those studies did not disentangle the specific impact of telomerase activation from telomere  
296 lengthening. The current absence of clear data showing increased ATP consumption associated with  
297 telomerase activity allows an alternative view that telomerase may modulate in return the efficiency  
298 of the cellular energy-generating machinery.

### 299 *The intriguing telomerase and mitochondrial relationship*

300 Interestingly, among the non-canonical cell functions of telomerase so far described (reviewed  
301 in Arndt & MacKenzie, 2016), one intriguing finding is the mitochondrial localization of the *TERT* –  
302 telomerase subunit, recurrently reported by independent research groups (Ahmed et al., 2008;  
303 Haendeler et al., 2009; Moslehi, DePinho, & Sahin, 2012). This appears to be of interest for studying  
304 the *TERT* specific role in the context of life-history trade-offs, since the mitochondria stands at the  
305 crossroads of the production of cell energy (*i.e.* ATP) and of deleterious by-products of cell respiration  
306 (*i.e.* reactive oxygen species (ROS)) (Criscuolo et al., 2005; Sahin & DePinho, 2012; Speakman et al.,  
307 2004). The first observation came from *TERT* deficient mice strains that showed compromised  
308 mitochondrial biogenesis through decreased mitochondrial content in somatic tissues, such as the  
309 heart and liver, as well as through decreased ATP mitochondrial synthesis (Sahin et al., 2011). The  
310 cellular pathways implicated in those negative effects have been clearly established. The lack of *TERT*  
311 expression leads to the suppression of the PGC activation pathway (Sahin et al., 2011) with cascading  
312 negative effects on gluconeogenesis, fatty acid oxidation or oxidative balance (Sahin & DePinho, 2012).  
313 Whether those changes are telomere-length dependent or due to non-canonical functions of *TERT*  
314 remains under debate. *TERT* was found to bind to genes coding for the complex I of the respiration  
315 chain, a major site of superoxide production, to improve respiratory chain activity (of complex I and of  
316 maximal respiration rate) and to protect mitochondrial DNA from oxidative stress (Haendeler et al.,  
317 2009). However, at least one study found higher levels of mitochondrial DNA damages due to hydrogen  
318 peroxide when *TERT* was re-localized within the mitochondria, and suggested that the mitochondrial  
319 – telomerase relationship was part of the oxidative stress pathway that triggers cell apoptosis (Santos,

320 Meyer, Skorvaga, Annab, & Van Houten, 2004). In addition, *TERC* *-/-* mice but which retain *TERT*  
321 expression, presented the same mitochondrial dysfunction as *TERT*-deficient mice, strongly suggesting  
322 a telomere length deficiency (and not a non-canonical) explanation (Sahin & DePinho, 2012; Strong et  
323 al., 2011). In the future, studying the effects of telomerase on mitochondrial functioning and then on  
324 the bioenergetics of cells needs to be extended in two ways. First, towards the study on how  
325 telomerase, mitochondrial dysfunction and signalization pathways well-known to control the energy  
326 trade-offs of organism ageing (*e.g.* IGF1 and mTOR) are interconnected (reviewed in Sahin & DePinho,  
327 2012). Whether the telomerase regulation of the mitochondrial functioning is direct or indirect is not  
328 of primary importance for evolutionary studies, but how telomerase may modulate those signalization  
329 pathways will uncover new sides of the individual variability in the growth and ageing trade-off.  
330 Second, we need to extend those studies to a broader range of species, starting with those that  
331 maintain telomerase activity after early-life development. As with telomeres, telomerase,  
332 mitochondria and IGF1 / mTOR pathways are highly conserved over evolution. Exploring how they  
333 differently interact in species that evolved variable growth rates, metabolism and lifespans would be  
334 highly valuable. For instance, telomerase activity is not found in dipterian insects (Sasaki & Fujiwara,  
335 2000), but whether non-canonical functions of telomerase play a role in individual fitness in those  
336 animals remains unexplored.

### 337 **The best models to start with: the *TERT* *-/-* knock-out animals**

338 *On longevity trade-off...*

339 Tackling the role of telomerase in regulating the trade-offs among cellular and life-history traits  
340 will be of paramount importance in the coming years. While it remains indescribably complex to  
341 decipher causality in telomere biology and ageing, one natural starting place is to examine how life-  
342 history traits like growth, reproduction and lifespan are combined in biological models that  
343 experimentally lack telomerase activity, such as the *TERT* or *TERC* *-/-* organisms (Anchelin et al., 2013;  
344 Blasco et al., 1997). Those knock-out models have provide us with some clues that need to be explained



345 in a life-history context. Up-to-date, only one study has indirectly addressed the variation of expression  
346 of telomerase and of *TERT* / *TERC* subunits in relation to lifespan evolution and extrinsic risks of  
347 mortality (Hartmann et al., 2009). In short (3 months) and longer-lived (6 months) killifish,  
348 *Nothobranchius furzeri*, *TERT* and *TERC* gene expression correlate with telomerase activity and are  
349 equally expressed in both strains, but increase with age in some tissues of the long-lived population.  
350 Since telomere ends erode with age in both strains, we may expect some non-canonical roles for  
351 telomerase subunits to play a role in lifespan in this animal. Using such populations of the same species,  
352 that have naturally evolved life-history trait differences due to ecological constraints (*i.e.* the short-lived  
353 killifish originates from temporary pools with eggs drying in the mud until the next generation, and so  
354 we short adult lifespan expectancy), we may extend our understanding of the impact of telomerase  
355 and telomerase subunits' expression to divergent evolution of fast pace of life (*i.e.* fast growth and  
356 high reproductive investment).

357         ...reproduction trade-off...

358         *TERC* *-/-* mice show progressive telomere shortening over generations, with reduced cell  
359 turnover, bone marrow failure, and reproductive impairment only in the sixth generation of  
360 homozygous mutants (*TERT* *-/-* mice presented similar phenotypes (Herrera et al., 1999; Strong et al.,  
361 2011) for consistent results in a mouse strain with a different genetic background). Telomerase-  
362 mutated *C. elegans* worms (*trt-1*) can survive the absence of full telomerase activity and show no  
363 particular deficiencies, apart from becoming sterile after few generations (Lackner & Karlseder, 2012).  
364 While being rather anecdotal for molecular and cell biologists focusing on lifespan, those studies  
365 conducted on very different species may say something about the control of reproduction by age  
366 signalling, but may also have missed how impaired telomerase activity may modulate the reproductive  
367 output of adults. If accumulation of DNA damage over generations is the main explanation for the late  
368 appearance of specific sterile phenotypes in knockout models, we may expect that reproduction, or  
369 any other activity either energy consuming or potentially stressful, would also be more detrimental for

370 the future reproductive and survival prospects of *TERT* *-/-* or *TERC* *-/-* breeders. Unfortunately, the  
371 original work on mice said nothing about the reproductive success of those mice (Blasco et al., 1997),  
372 while mutated worms did show a decrease in brood size (Lackner, Raices, Maruyama, Haggblom, &  
373 Karlseder, 2012). Additional telomerase mutants like *TERT* *-/-* zebrafish (in which telomerase is active  
374 through life and present in the ovary even in infertile old females) develop normally until adulthood  
375 (Anchelin et al., 2013) and start with normal clutch size and viability (*i.e.* until 1 year-old). However,  
376 *TERT* *-/-* zebrafishes then present a fast senescence and premature sterility (Henriques & Ferreira,  
377 2012). Interestingly in this study, *TERT* *-/+* and *TERT* *+/+* zebrafish increased their reproductive  
378 performances after 1 year, while the female *TERT* *-/-* presented a reduced number of eggs *per* spawn  
379 (Anchelin et al., 2013). Still, there were no detailed data on how, for instance, the heterozygote strain  
380 may suffer from a lowered reproductive success than the control one. Such a question remains a key  
381 point to evaluate the putative telomerase – fitness links. Again, given that *TERT* has been found to have  
382 non-canonical roles in inducing neutropenia in zebrafish (Alcaraz-Pérez et al., 2014), it is unknown  
383 whether this could also modulate reproductive success *via* gamete viability (see Anchelin et al., 2013  
384 for deleterious effect on sperm).

385           *...or immune trade-off.*

386           Since cell proliferation is one of the active arms of immunity, a last obvious context where  
387 telomerase impact should be evaluated in knock-out models relates to the cost of mounting an  
388 immune response. Telomerase seems to be involved in replicative capacity of immune cells (Allsopp,  
389 Cheshier, & Weissman, 2002) and *TERC* *-/-* mice show a reduced spleen size with a reduced rate of  
390 proliferation of either B and T lymphocytes following immune challenge (Blasco, 2002). In addition, a  
391 higher proportion of neutrophils has been recorded (Herrera et al., 1999) (maybe as a compensatory  
392 response to lymphocyte weakness) and macrophages of *TERC* *-/-* mice are characterized by an “old-  
393 phenotype” with reduced proliferative capacity and higher intracellular ROS concentrations (Sebastián  
394 et al., 2009). As neutrophils and macrophages are central in the set-up and control of the inflammatory

395 and innate immune responses (Desai, Grolleau-Julius, & Yung, 2010), evaluating how *TERC* -/-  
396 individuals react to inflammatory challenges commonly used in ecophysiological studies (*i.e.*  
397 lipopolysaccharide injection) will be of interest, both in terms of energy expenditure of collateral  
398 oxidative damage, and ultimately of future reproductive and survival prospects.

### 399 **Conclusion: there is a lot to gain in studying telomerase for evolutionary biologists**

400 Our present knowledge of the role of telomerase in modulating cell ageing, bioenergetics or  
401 signalization pathways (all mechanisms underlying the individual phenotype and ultimately its life-  
402 history traits such as immunity, growth or reproductive rates) is largely lacking. Using knock-out  
403 models of telomerase genes has provided us with a unique tool to study how the combination of  
404 physiological and life history traits is likely to be altered when compared to wild-type strains. Of course,  
405 heterozygotes of mutated genes (*TERT* -/+ or *TERC* -/+) will be more valuable as they will take us  
406 beyond the on/off (*i.e.* sterile/fertile) phenotypes. In addition, the study of telomerase must be  
407 extended to a broader range of species in non-models animals like birds, fish, mammals or insects,  
408 where descriptive studies on change in telomerase activity with age or tissue have already paved our  
409 way (e.g. (Hartmann et al., 2009; Jemielity et al., 2007; Koubová et al., 2019; McAloney, Silverstein,  
410 Modiano, & Bagchi, 2014; O'Hare & Delany, 2005; Wang, McAllan, & He, 2011). Adding original data  
411 on, more particularly, the pleiotropic action of canonical and non-canonical activities of telomerase,  
412 and exploring how they may have co-evolved with particular life-history traits form our next scientific  
413 objective.

414

### 415 **Acknowledgements**

416 **Data accessibility.** This article has no additional data.

417 **Competing interests.** We declare we have no competing interests.

418 **Funding.** We received no funding for this study

419 **Author contributions**

420 S.S. and F.C. conceived the study. S.S, F.C., F.H. and S.Z. wrote the text. All authors commented on  
421 drafts of the manuscript.

422

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761 **Data accessibility statement**

762

763 **Author contributions**

764 **Supplementary Information**

765 Supplementary Table 1: XXXX

766