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

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Abstract

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

KEYWORDS
immunity, life history, reproductive effort, telomeres

1 | INTRODUCTION

The burgeoning field of telomere dynamics has added new perspectives and directions to the study of ecology and evolutionary biology. The interest is testament to the fact that telomeres represent an intriguing biomarker that provides a link between individual physiology, life history, and fitness. As the field has developed, so too has the realisation that the maintenance of telomeres is at the centre of this balance. Studies so far, both experimental (Herborn et al., 2014; Nettle et al., 2015) and correlational (Hoelzl et al., 2016; Turbill et al., 2012), indicate that telomere restoration is costly (reviewed in Young, 2018). However, the mechanisms underlying telomere maintenance remain

elusive and evolutionary biologists have turned to clinical research for answers. The obvious candidate as regulator within the system is the enzyme complex telomerase. Telomerase comprises an RNA component (*TERC*) and a catalytic protein domain (TERT) essential for the reverse transcriptase activity of the enzyme (see Box 1). Telomerase is known to catalyse the elongation and maintenance of telomeres by adding telomeric repeats to the chromosomal ends and thus counteracting losses that occur d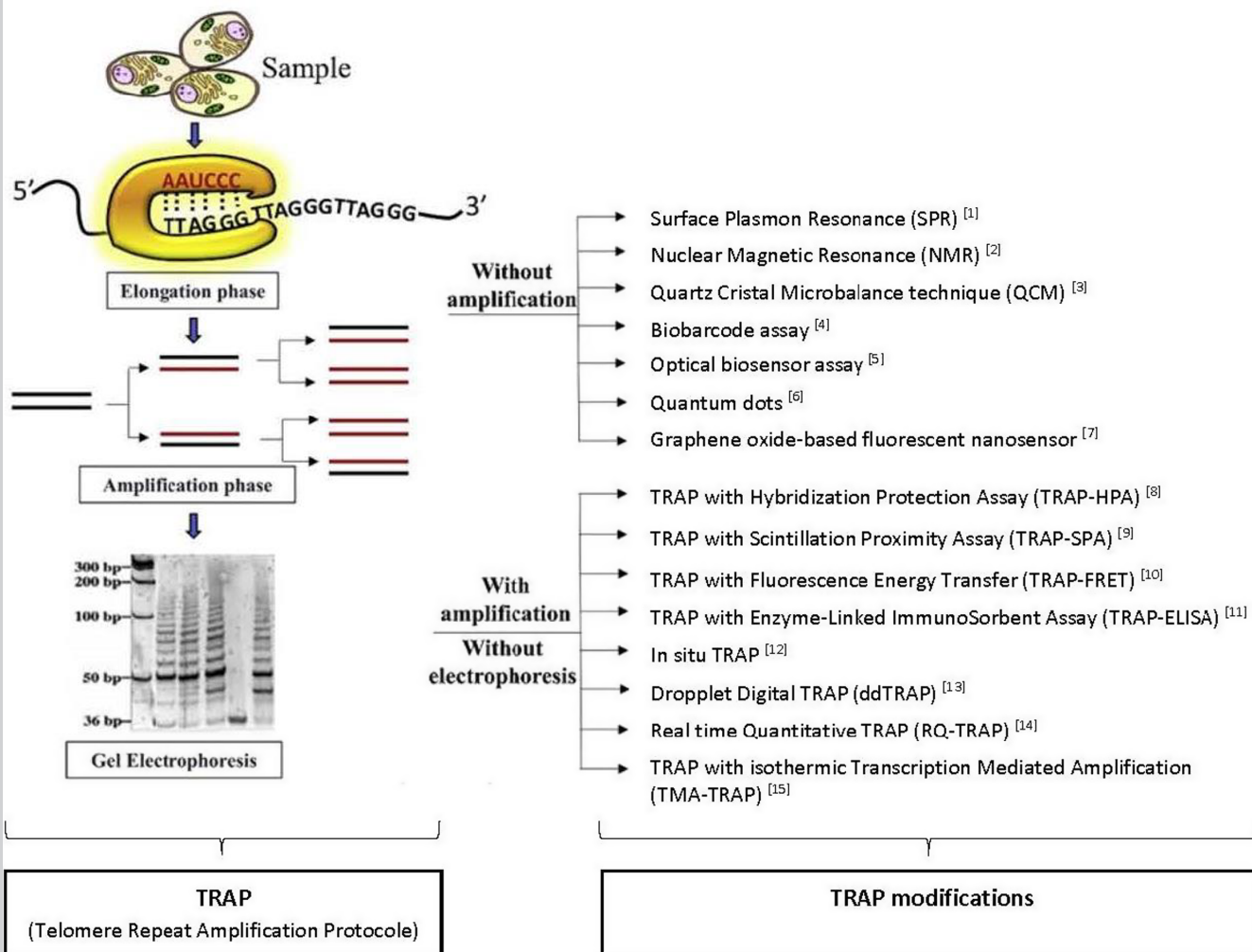uring end replication and oxidative stress (Harley et al., 1990; von Zglinicki, 2002). For most organisms, telomerase is not active throughout all life stages and across all tissue types (Haussmann et al., 2004; Lingner et al., 1997; Logeswaran et al., 2021; Wright et al., 1996), strongly suggesting trade-offs between life history stages such as growth, maintenance and reproduction. As a potential regulator of these trade-offs, measurement of telomerase activity (TA) is of increasing interest for evolutionary 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 (see figure 1 and description in Sandin & Rhodes, 2014).

- 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 et al., 2015), CTE is necessary for stable binding of the enzyme to the DNA (Hossain et al., 2002), TRBD binds the RNA-template (Lai et al., 2001) and RT is the catalytic domain (Dey & Chakrabarti, 2018; Lingner et al., 1997).

- *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 et al., 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 et al., 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 et al., 2011). In *TERC*, the arrangement and composition of elements (PK, TBE, etc) is variable between taxa. On the sequence and structure level of these different elements, the variability between taxonomic groups is even more pronounced (Logeswaran et al., 2021).

- Trafficking of TERT and *TERC* between nucleus and cytoplasm is not fully understood. In yeast (*Saccharomyces cerevisiae*), the assembly of the telomerase holoenzyme probably 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 TA (reviewed in Gallardo & Chartrand, 2008). TA 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 et al., 2004; Zvereva et al., 2010).

- A study by Florell et al. (2001) and Schmidt et al. (2016) reveals that telomerase diffuses throughout the nucleoplasm and that the enzyme frequently binds TPP1 (a protein of the shelterin complex) at telomeres. The Shelterin complex is formed by several proteins and protects the chromosomal ends from degradation (e.g., de Lange, 2005). The TPP1 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 Schrumpfová 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, TA is usually detected in all early developmental stages. After organogenesis, TA is restricted to proliferating stem cells, while it is downregulated in somatic cells (reviewed in Schrumpfová et al., 2019). In rodents, the tendency to downregulate somatic TA is body mass dependent, where species of weight smaller than 1 kg (e.g., mice) keep TA throughout their life, while larger animals downregulate telomerase in somatic tissues (likely to reduce the risk of cancer; Seluanov et al., 2007).

BOX 2   Measurement of telomerase activity: previous studies and methods employed

For more than a decade, evolutionary ecologists have focussed on telomeres (Autexier & Lue, 2006; Haussmann & Vleck, 2002),

Techniques employed to quantify telomerase activity (TA)

Sample

AAUCCC
5' ... TTAGGG TTAGGGTTAGGG — 3'

Elongation phase

Amplification phase

300 bp
200 bp
100 bp
50 bp
36 bp

Gel Electrophoresis

Without amplification
→ Surface Plasmon Resonance (SPR) [1]
→ Nuclear Magnetic Resonance (NMR) [2]
→ Quartz Cristal Microbalance technique (QCM) [3]
→ Biobarcode assay [4]
→ Optical biosensor assay [5]
→ Quantum dots [6]
→ Graphene oxide-based fluorescent nanosensor [7]

With amplification
Without electrophoresis
→ TRAP with Hybridization Protection Assay (TRAP-HPA) [8]
→ TRAP with Scintillation Proximity Assay (TRAP-SPA) [9]
→ TRAP with Fluorescence Energy Transfer (TRAP-FRET) [10]
→ TRAP with Enzyme-Linked ImmunoSorbent Assay (TRAP-ELISA) [11]
→ In situ TRAP [12]
→ Dropplet Digital TRAP (ddTRAP) [13]
→ Real time Quantitative TRAP (RQ-TRAP) [14]
→ TRAP with isothermic Transcription Mediated Amplification (TMA-TRAP) [15]

TRAP
(Telomere Repeat Amplification Protocole)

TRAP modifications

followed by a shift of focus to TA (Autexier & Lue, 2006; Haussmann et al., 2007), in order to better understand individual variability in lifespan (Haussmann et al., 2005). However, evaluations of TA are not a simple prospect for most of the species of ecological interest. Logistical constraints and/or specific expertise requirements are the major challenges. For instance, storage and pretreatment of samples to preserve TA is inherently complicated because of the nature of this enzyme itself (1) (see Box 1—telomerase RNA component—(Florell et al., 2001; Shippen-Lentz & Blackburn, 1990; Theimer & Feigon, 2006; Zvereva et al., 2010). Thus the development of reliable TA assessment methodologies is essential to allow research in evolutionary biology to develop and understand covariation of age- or tissue-related TA with animal life-histories. This schematic representation shows the most common methods employed to measure TA in human and cancer research. The methods are presented following two main categories: (2) detection of telomerase products and (3) amplification of these products (TRAP-based methods). For method-based references [1] to [15] see Supporting Information materials. Figure is adapted from Mensà et al. (2019).

Senescence is defined in evolutionary biology as the relaxation of natural selection at old-ages, attributed to the reduced fitness contribution of older breeders due to poor reproductive performances and survival rates (Gaillard & Lemaître, 2020). Williams' theory of senescence stipulates that it should be found in all living organisms that have, among other prerequisites (Gaillard & Lemaître, 2017), genes that will be beneficial early in life and detrimental later on (i.e., the antagonistic pleiotropy of ageing, Williams, 1957). At a

more mechanistic level, senescence, which promotes cell division arrest and apoptosis, could be considered as a cell response to ageing stress, with both positive and negative impacts on individual fitness. For instance, cell senescence also promotes regular tissue repair, and protects against cell immortalization and cancer, while at the same time triggering proinflammatory or tissue degeneration processes (reviewed in Autexier & Lue, 2006; Campisi, 2013). Because telomeres and telomerase stand at the crossroad of these cell mechanisms, we propose here that the study of telomerase will benefit from being considered in the context of its pleiotropic effects on individual fitness and through its modulation of life-history trade-offs. To do so, we highlight that (i) telomerase genes vary in sequences and expression, (ii) telomerase may act via nontelomere-related pathways, (iii) telomerase may have pleiotropic effects due to dynamic changes in expression and thus mediate trade-offs among traits. We then address what could be the costs associated to telomerase activation, and propose a few avenues for studying the putative telomerase—fitness link.

2 | THE ECOLOGICAL LINK TO TELOMERASE

Early studies of telomere dynamics in non-model organisms reported individual differences in telomere attrition related to age (Aubert, 2014), birth order (Noguera et al., 2016), birth timing (Eisenberg et al., 2012), early life stress (Cram et al., 2017; Price et al., 2013), reproductive output (Beaulieu et al., 2011; Monaghan & Haussmann, 2006), rewarming during hibernation (Hoelzl, Smith, et al., 2016), and habitat suitability (Angelier et al., 2013) among others. After this initial interest in telomeres as a reliable biomarker for stress and biological ageing, evidence began to emerge that, in some species at least, telomere length could be actively restored or even elongated (Foley et al., 2018; Haussmann et al., 2003; Hoelzl, Smith, et al., 2016). Despite initial scepticism, further studies confirmed active telomere maintenance across multiple species. TA was considered the most likely mechanism responsible for telomeric restoration and once again ecological researchers borrowed from clinical fields and cancer research to find the best way to measure TA in an ecological setting (see Box 2). Subsequent work has implicated an association between TA levels and telomere dynamics in a broad range of taxa. Mu et al. (2015) found a link between seasonal leaf growth, telomere length and TA in the Chinese Pine (*Pinus tabulaeformis*). Interestingly, telomere length increased with temperature throughout the year whereas TA showed a negative association, suggesting a potential lag between stimulus and response. A strong association between TA and telomere length was observed in multiple strains of zebra fish (*Danio rerio*) with a marked drop in both detected in older individuals (Anchelin et al., 2011). In a follow-up experimental study (Anchelin et al., 2013), telomerase-knockdown zebra fish showed premature ageing and reduced lifespan in the first generation. Restoration of TA in the second generation led to rescue of telomere length and survival confirming the causal role

of telomerase in the association. Likewise, Hatakeyama et al. (2016) demonstrated in Japanese medaka (*Oryzias latipes*) that telomere attrition and restoration are linked to growth and TA respectively, and that a critical loss of telomere homeostasis is associated with mortality. Recently, an intriguing link with embryo corticosterone levels was shown in yellow-legged gulls, *Larus michahellis* (Noguera et al., 2020). Hatchlings from corticosterone–injected eggs were shown to have both higher levels of TA and longer telomeres. Telomerase was also shown to induce the synthesis and repair of TTAGG-telomeric repeats in Lepidoptera (Gong et al., 2015) and this association seems to be widespread in most orders of insects (Korandová et al., 2014). Combined, these studies largely demonstrate that telomere length and telomerase are closely coupled as expected based on in vitro studies and tightly regulated by environmental factors.

Crucially though, many studies have not detected the expected link between telomere maintenance and TA (see citations in Table S1). Clinical studies in humans and experimental work show that in many conditions, telomere length is uncoupled from TA (Xie et al., 2015) and that telomerase acts in diverse functions at the cellular and physiological levels (Haendeler et al., 2009; de Punder et al., 2019; Sahin & DePinho, 2012; Saretzki, 2009). Work in honeybees (*Apis mellifera*) also could not find a link between telomere length and TA, with the latter being regulated in a developmental and caste-specific manner (Korandová & Čapková Frydrychová, 2015; see also Koubová et al., 2019). The inconsistent findings from studies of the telomere—telomerase relationship raise questions about the ubiquity of a single telomere repair mechanism but, perhaps more interestingly, also suggests alternative roles for telomerase and its subunits in life history trade-offs. Resolving the links in these trade-offs promises to open the door to exciting avenues of research in ecology and evolutionary biology.

3 | LINKING LIFE HISTORY TRADE-OFFS AND INDIVIDUAL FITNESS WITH TELOMERASE?

The essential requirement for a biological variable to play a role in evolution is to show variation among individuals in a population (along with transmission to the next generation and conferring some fitness effects). Telomerase is formed via the assemblage of two main subunits coded by highly conserved genes (*TERT*, *TERC*). The eutherian *TERT* genes are characterized by a recent evolution history from reptile-like forms, such as the platypus *TERT* ortholog (Hrdličková et al., 2012) and have thus emerged with the ancestral mammals. More importantly, polymorphisms of those genes do exist in humans, their discovery originating from the study of telomere diseases such as congenital dyskeratosis (Savage et al., 2008; Vulliamy et al., 2001). In those patients, the RNA component of the telomerase (*TERC*) and an associated regulating protein (dyskerin, *DCK1* gene) are mutated, leading to defective telomere maintenance by telomerase and premature ageing phenotypes (Marrone & Dokal, 2004). Human *TERT* mutations were also found to be associated with

different, rather late-life, diseases (Calado & Young, 2012), and *TERT* single nucleotide polymorphisms have been identified in humans, often associated with an increase in cancer risks (Rafnar et al., 2009, together with shortened telomeres in this study), or bone marrow failure (Yamaguchi et al., 2005). Enhanced TA is found in more than nine out of 10 cancer cells (Shay & Bacchetti, 1997). So how could telomerase defects enhance cancer risks? Causality is thought to relate to the capacity of haematopoietic cells (and then immune cells) to upregulate telomerase when activated. Given that the level of *TERT* expression may be the rate-limiting mechanisms of control of TA (Counter et al., 1998), *TERT* deleterious mutations may shorten telomere length of leucocytes, and then decrease the ability of the immune system to survey cell immortalization and eliminate cancer cells (Calado & Young, 2012). Therefore, focusing on the individual variation in *TERT* expression and TA in white blood cells, and characterising how it may modulate immunosenescence (de Punder et al., 2019), may provide the first insights into the telomerase—fitness relationship. In addition, telomerase polymorphisms have been previously associated with noncancer diseases, with significantly modified soma protective effects (Snetselaar et al., 2018). *TERC* variants were shown to have an opposite effect on longevity in humans (Soerensen et al., 2012), thereby suggesting that telomerase mutations may carry putative fitness benefits and costs.

3.1 | Telomerase: a two-headed coin with telomere length and nontelomere length sides

The studies cited above mainly explain their results based on the protective action of telomerase on telomeres, which is of prime interest for biologists seeking telomerase—fitness relationships but also renders difficult any conclusion on telomerase effects per se. However, noncanonical effects of telomerase do exist (i.e., not mediated by the maintenance of telomere length) and should be taken into account to explore further the potential fitness benefits provided by this enzyme. Insights for the putative importance of these noncanonical roles in shaping life-histories were recently illustrated in an interspecific comparison study of *TERT* sequences and expressions (Lai et al., 2017). Highly variable patterns of alternative splicing of the catalytic subunit *TERT* was described even in closely related species, suggesting that different functions of telomerase may have evolved in relation to lineage specific life histories (Lai et al., 2017). However, to date, the nature of those non-canonical roles (and their control by alternative splicing) are almost exclusively studied in humans and seem restricted to a few main impacts of *TERT* expression: mitochondrial functioning, enhanced cell proliferation (via the activation of growth factor signalisation pathway), control of the gene expression (not only oncogenes, e.g., immune genes) and regulation of TA during early-life development (de Punder et al., 2019; Smith et al., 2003; Ulaner et al., 1998; Xiang et al., 2000). One first research question to be addressed could be related to the stress-induced colocalisation of telomerase into the mitochondria when human cells are exposed to a mild oxidative stress event (Ahmed et al., 2008).

Even if we need additional aspects of TA or *TERT* to be better described (such as nontelomeric DNA maintenance, Gorbunova et al., 2002), the implication of such a telomerase-dependent response in explaining interindividual differences to environmental stress would be of broad interest for evolutionary biologists. They suggest that either due to genetic variance or post-transcriptional regulation, individuals may react differently to environmental factors, for which the physiological response needs the activation of *TERT* expression and/or TA. One intriguing observation was made in planarian worms, an animal showing tissue regeneration capacity and asexual or sexual reproduction. In this model, alternative splicing of *TERT* seems to regulate the mechanisms of telomere elongation either in the somatic cells during fission (asexual reproduction) or in the germline cells during sexual reproduction (Tan et al., 2012). Together with similar observations in colonial ascidians or urochordate animals, it suggests that variation in telomerase expression has coevolved with specific life-history traits like mode of reproduction (Lai et al., 2017), extreme lifespan or somatic regeneration capacity (Tan et al., 2012 and herein cited references).

3.2 | Tackling the telomerase—fitness link from its pleiotropic nature

We have briefly introduced so far that the role of telomerase may go beyond its accepted contribution to the maintenance of telomere length (noncanonical impacts of the *TERT* or *TERC* subunits), which may be associated with species' traits. However, to tackle the telomerase—fitness hypothesis, we may also reconsider the question through the prism of its main period of high activity (at least in most birds and mammals), early-life development, and of its primary role in cell replication (Gorbunova & Seluanov, 2003). Could telomerase have a dual and age-related impact on fitness, via individual health and lifespan components? By favouring developmental processes and cell renewal in early life, do TA and noncanonical *TERT*/*TERC* expression have positive effects on individual reproductive output, while leading to increased cancer risks in later life? When thinking of such pleiotropies of telomerase and fitness relationships, the first objective of future studies should be to evaluate how early and late-life expression of telomerase may modulate individual fitness at young, reproductive and post-reproductive adult stages (i.e., in accordance with predictions of the antagonistic pleiotropic ageing theory). Since early-life consequences remain difficult to evaluate and need long-term data sets of individual life trajectories, focusing on animal models in which TA regularly rise naturally during their life cycle would be an interesting option. For instance, hibernator species are known to reactivate telomerase at the start of their active life cycle (Hoelzl, Cornils, et al., 2016; Hoelzl, Smith, et al., 2016; Turbill et al., 2013), and determining how the variance in activity or in body and intracellular localisation explain subsequent individual performance in reproduction and survival would be relatively easy to achieve. Nevertheless, *TERT* alternative splicing is recognized as

being involved in modulating TA during development in humans, but only its consequence in terms of telomere length has so far been evaluated (reviewed in Shay & Wright, 2019). This question needs to be re-evaluated in biological models where individual health and fitness can be measured over life. Along the same lines, the pleiotropies of noncanonical effects should also be studied. In fact, *TERT* ectopic expression in mice fibroblasts triggers cell growth by repressing growth-inhibiting pathways (Geserick et al., 2006). This suggests that individual variation in *TERT* expression may underline individual variation in growth rates, and thereby potentially contribute to the growth—lifespan trade-off (Metcalfe & Monaghan, 2003).

3.3 | Tackling the telomerase—fitness link from its dynamic nature

An important second way of studying telomerase in an evolutionary context will be to search for acute TA or *TERT* expression in response to stress under natural conditions. Telomerase is mainly described as active during embryogenesis and early-life in most animals (Bekaert et al., 2004; Prowse & Greider, 1995), and then is shut down to avoid cancer-risks (Shay & Wright, 2019). However, this general view has been regularly amended by data collected on several taxa, suggesting that TA could be maintained at adulthood in certain conditions. For instance, beside the maintained TA in the soma of insects, lizards or fish (reviewed in Gomes et al., 2010), repression of TA was not found as an unalterable characteristic in mammals and birds. TA actually covaries (negatively) with species body mass in rodents (Gorbunova & Seluanov, 2009) and life-long TA characterizes some long-lived small seabirds (Haussmann et al., 2007), while its expected positive impact on telomere length was described in hibernating (Hoelzl, Cornils, et al., 2016; Turbill et al., 2013) as well as in non-hibernating species (Criscuolo et al., 2020; Fairlie et al., 2016). The latter studies highlighted that telomerase may be turned-on at certain life stages, probably in some particular cells (e.g., stem or haematopoietic cells) and raises the question of whether telomerase forms part of the response-repertoire an organism possesses to face stress (Beery et al., 2012), or which may constrain adaptive abilities (Choi et al., 2008). Given the ability of immune cells to express high TA when activated (Bodnar et al., 1996; Hiyama et al., 1995; Yamada et al., 1996), future studies would benefit from a focus on immune cells, and how changes in TA (i.e., increases) vary at the individual level in response to immune challenge and what are the collateral effects on other somatic tissues. Still, while exploratory, those studies will probably uncover only part of the global picture as they will focus solely on the dynamics of TA. Adding the noncanonical functions of telomerase via the characterization of *TERT / TERC* dynamics of expression over age and in response to stress is vitally important to establish how telomerase may affect individual fitness or bestow individuals with specific life-history trait associations.

3.4 | Telomerase as a mediator of the reproductive trade-off

The different age-related risks of TA have so far been conceptualized based on our present knowledge of telomerase implication in cell-growth or renewal processes (Morrison et al., 1996) and in cell immortalization and the associated cancer risk (Tian et al., 2018). However, the risk has never been considered in relation to the cost of reproduction in adults, neither for TA nor at the level of subunit expression and of their noncanonical effects. When studying the cost of reproduction, defined as decreased future reproductive and survival rates (Stearns, 1992), one may consider telomerase as one of the pleiotropic regulators that may affect adult breeders current success (via the maintenance of telomere length) and their future prospects (via the noncanonical *TERT/TERC* protective effects, see below). Because the cost of reproduction may be mediated through telomere erosion or other cell defects of the adult soma and of gametes, using telomerase as the response variable will extend our understanding of reproductive costs beyond the "Y model" of energy allocation between reproduction and lifespan (reviewed in Harshman & Zera, 2007). Such a possibility has been illustrated in a single cell-organism, yeast, where a role for telomerase in response to DNA replication stress, but also in additional maternal cell ageing, was highlighted (Xie et al., 2015). Such a telomerase-based fitness component may also relate to the regulation by telomerase of stem cells capacity to be mobilized and punctually proliferate (Calado & Chen, 2006), thereby modulating cell renewal rate and organ performance in breeders. Again, focusing on immune cells, as it is the somatic tissue where TA is more likely to be recorded, seems the best alternative if we are to evaluate how telomerase may mediate the ageing outcomes of reproduction (Schulenburg et al., 2009).

4 | EVIDENCE FOR A COST OF TELOMERASE ACTIVATION

The first cost to consider for TA in an evolutionary biology sense is energetic. A previous review on the role of telomeres in the evolution of life-history traits underlined the importance (and lack) of obtaining accurate data on how costly it is for a cell, and thereby for an organism, to maintain telomere length (and thus indirectly in activating telomerase, Young, 2018). Ecophysiological studies have suggested that maintenance of telomere length may be interpreted as energetically costly since, for instance, food supply during bad years allowed better telomere maintenance in free-living edible dormice (*Glis glis*; Hoelzl, Cornils, et al., 2016; Nowack et al., 2019). Still, those studies did not disentangle the specific impact of telomerase activation from telomere lengthening. The current absence of clear data showing increased ATP consumption associated with TA allows an alternative view that telomerase may modulate in return the efficiency of the cellular energy-generating machinery.

4.1 | The intriguing telomerase and mitochondrial relationship

Interestingly, among the noncanonical cell functions of telomerase so far described (reviewed in Arndt & MacKenzie, 2016), one intriguing finding is the mitochondrial localization of the *TERT*—telomerase subunit, recurrently reported by independent research groups (Ahmed et al., 2008; Haendeler et al., 2009; Moslehi et al., 2012). This appears to be of interest for studying the *TERT* specific role in the context of life-history trade-offs, since the mitochondria stands at the crossroads of the production of cell energy (i.e., ATP) and of deleterious byproducts of cell respiration (i.e., reactive oxygen species (ROS); Criscuolo et al., 2005; Sahin & DePinho, 2012; Speakman et al., 2004). The first observation came from *TERT* deficient mice strains that showed compromised mitochondrial biogenesis through decreased mitochondrial content in somatic tissues, such as the heart and liver, as well as through decreased ATP mitochondrial synthesis (Sahin et al., 2011). The cellular pathways implicated in those negative effects have been clearly established. The lack of *TERT* expression leads to the suppression of the peroxisome proliferator-activated receptor gamma, coactivator 1 (PGC) activation pathway (Sahin et al., 2011) with cascading negative effects on gluconeogenesis, fatty acid oxidation or oxidative balance (Sahin & DePinho, 2012). Whether those changes are telomere-length dependent or due to noncanonical functions of *TERT* remains under debate. *TERT* was found to bind to genes coding for the complex I of the respiratory chain, a major site of superoxide production, to improve respiratory chain activity (of complex I and of maximal respiration rate) and to protect mitochondrial DNA from oxidative stress (Haendeler et al., 2009). However, at least one study found higher levels of mitochondrial DNA damages due to hydrogen peroxide when *TERT* was relocalized within the mitochondria, and suggested that the mitochondrial—telomerase relationship was part of the oxidative stress pathway that triggers cell apoptosis (Santos et al., 2004). In addition, *TERC* −/− mice which retain *TERT* expression, presented the same mitochondrial dysfunction as *TERT*-deficient mice, strongly suggesting a telomere length deficiency (and not a noncanonical) explanation (Sahin & DePinho, 2012; Strong et al., 2011). In the future, studying the effects of telomerase on mitochondrial functioning and then on the bioenergetics of cells needs to be extended in two ways. First, towards the study on how telomerase, mitochondrial dysfunction and signalling pathways well-known to control the energy trade-offs of organism ageing (e.g., IGF1 and mTOR) are interconnected (reviewed in Sahin & DePinho, 2012). Whether the telomerase regulation of the mitochondrial functioning is direct or indirect is not of primary importance for evolutionary studies, but how telomerase may modulate those signalling pathways will uncover new sides of the individual variability in the growth and ageing trade-off. Second, we need to extend those studies to a broader range of species, starting with those that maintain TA after early-life development. As with telomeres, telomerase, mitochondria and IGF1/mTOR pathways are highly conserved over evolution. Exploring how they differently interact in species that evolved variable growth rates, metabolism and lifespans would be highly valuable. For instance, TA is not found in dipterian insects (Sasaki & Fujiwara, 2000), but whether noncanonical functions of telomerase play a role in individual fitness in those animals remains unexplored.

5 | THE BEST MODELS TO START WITH: *TERT* −/− KNOCK-OUT ANIMALS

5.1 | On longevity trade-off...

Tackling the role of telomerase in regulating the trade-offs among cellular and life-history traits will be of paramount importance in the coming years. While it remains indescribably complex to decipher causality in telomere biology and ageing, one natural starting place is to examine how life-history traits like growth, reproduction and lifespan are combined in biological models that experimentally lack TA, such as the *TERT* or *TERC* −/− organisms (Anchelin et al., 2013; Blasco et al., 1997). Those knock-out models provide us with some clues that need to be explained in a life-history context. To date, only one study has indirectly addressed the variation of expression of telomerase and of *TERT/TERC* subunits in relation to lifespan evolution and extrinsic risks of mortality (Hartmann et al., 2009). In short (3 months) and longer-lived (6 months) killifish, *Nothobranchius furzeri*, *TERT* and *TERC* gene expression correlate with TA and are equally expressed in both strains, but increase with age in some tissues of the long-lived population. Since telomere ends erode with age in both strains, we may expect some noncanonical roles for telomerase subunits to play a role in lifespan in this animal. Using such populations of the same species, that have naturally evolved life-history trait differences due to ecological constrains (i.e., short-lived killifish originate from temporary pools with eggs drying in the mud until the next generation, thus exhibiting short adult lifespan expectancy), we may extend our understanding of the impact of telomerase and telomerase subunit expression to divergent evolution of fast pace of life (i.e., fast growth and high reproductive investment).

5.2 | ...reproduction trade-off...

TERC −/− mice show progressive telomere shortening over generations, with reduced cell turnover, bone marrow failure, and reproductive impairment only in the sixth generation of homozygous mutants (*TERT* −/− mice presented similar phenotypes (Herrera et al., 1999; Strong et al., 2011) for consistent results in a mouse strain with a different genetic background). Telomerase-mutated *C. elegans* worms (*trt*-1) can survive the absence of full TA and show no particular deficiencies, apart from becoming sterile after few generations (Lackner & Karlseder, 2013). While being rather anecdotal for molecular and cell biologists focusing on lifespan, those studies conducted on very different species may say something about the control of reproduction by age signalling, but may also have missed how impaired TA may modulate the reproductive output of adults. If accumulation of DNA damage over generations is the main explanation for the late appearance of specific sterile phenotypes

in knockout models, we may expect that reproduction, or any other activity either energy consuming or potentially stressful, would also be more detrimental for the future reproductive and survival prospects of TERT −/− or TERC −/− breeders. Unfortunately, the original work on mice said nothing about the reproductive success of those mice (Blasco et al., 1997), while mutated worms did show a decrease in brood size (Lackner et al., 2012). Additional telomerase mutants like TERT −/− zebrafish (in which telomerase is active through life and present in the ovary even in infertile old females) develop normally until adulthood (Anchelin et al., 2013) and start with normal clutch size and viability (i.e., until 1-year-old). However, TERT −/− zebrafishes then present a fast senescence and premature sterility (Henriques & Ferreira, 2012). Interestingly in this study, TERT −/+ and TERT +/+ zebrafish increased their reproductive performances after 1 year, while the female TERT −/− presented a reduced number of eggs per spawn (Anchelin et al., 2013). Still, there were no detailed data on how, for instance, the heterozygote strain may suffer from a lowered reproductive success than the control one. Such a question remains a key point to evaluate the putative telomerase—fitness links. Again, given that TERT has been found to have noncanonical roles in inducing neutropenia in zebrafish (Alcaraz-Pérez et al., 2014), it is unknown whether this could also modulate reproductive success via gamete viability (see Anchelin et al., 2013 for deleterious effect on sperm).

5.3 | ...or immune trade-off

Since cell proliferation is one of the active arms of immunity, a last obvious context where telomerase impact should be evaluated in knockout models relates to the cost of mounting an immune response. Telomerase seems to be involved in replicative capacity of immune cells (Allsopp et al., 2002) and TERC −/− mice show a reduced spleen size with a reduced rate of proliferation of either B and T lymphocytes following immune challenge (Blasco, 2002). In addition, a higher proportion of neutrophils has been recorded (Herrera et al., 1999; maybe as a compensatory response to lymphocyte weakness) and macrophages of TERC −/− mice are characterized by an "old-phenotype" with reduced proliferative capacity and higher intracellular ROS concentrations (Sebastián et al., 2009). As neutrophils and macrophages are central in the set-up and control of the inflammatory and innate immune responses (Desai et al., 2010), evaluating how TERC −/− individuals react to inflammatory challenges commonly used in ecophysiological studies (i.e., lipopolysaccharide injection) will be of interest, both in terms of energy expenditure of collateral oxidative damage, and ultimately of future reproductive and survival prospects.

6 | TERT POLYMORPHISMS AND EXPRESSION DIFFERENCES WITHIN STUDY POPULATIONS

While knockout models offer an attractive starting point for examining telomerase effects, an alternative and perhaps parallel approach is to screen for polymorphisms and expression differences in TERT among individuals. Many ecological studies focus on the underlying causes and effects of oxidative stress particularly with respect to important fitness related parameters (e.g., reproduction, survival, longevity, immune status). The exploration of telomere dynamics has been a natural extension of these studies (reviewed above). A further focus could now be investigations of variation in telomerase critical genes at the genomic level and how that links with the underlying telomerase modulation and fitness effects. The explosion of next-generation sequencing technologies paves the way for studies interested in genomic polymorphisms and their phenotypic effects. Much can be adopted from human studies that have searched for similar links in relation to health and disease (Calado & Young, 2012; Rafnar et al., 2009; Savage et al., 2008; Vulliamy et al., 2001). Screening of entire populations for variation at a handful of candidate genes is now feasible for most labs. Linking TERT/TERC related polymorphisms to TA and other phenotypic differences should be a relatively simple add-on to many studies, particularly for well-studied captive and long-term population studies.

7 | CONCLUSION: THERE IS A LOT TO GAIN IN STUDYING TELOMERASE FOR EVOLUTIONARY BIOLOGISTS

Our present knowledge of the role of telomerase in modulating cell ageing, bioenergetics or signalization pathways (all mechanisms underlying the individual phenotype and ultimately its life-history traits such as immunity, growth or reproductive rates) is largely lacking. Using knockout models of telomerase genes has provided us with a unique tool to study how the combination of physiological and life history traits is likely to be altered when compared to wild-type strains. Of course, heterozygotes of mutated genes (TERT −/+ or TERC −/+) will be more valuable as they will take us beyond the on/off (i.e., sterile/fertile) phenotypes. In addition, the study of telomerase must be extended to a broader range of species in non-models animals such as birds, fish, mammals or insects, where descriptive studies on change in TA with age or tissue have already paved our way (e.g., Hartmann et al., 2009; Jemielity et al., 2007; Koubová et al., 2019; McAloney et al., 2014; O'Hare & Delany, 2005; Wang et al., 2011). Measurement of TA in such studies should be a natural next step via the use of already available and easily adaptable methods (e.g., TRAP assays, Mensà et al., 2019). Adding original data on, more particularly, the pleiotropic action of canonical and noncanonical activities of telomerase, and exploring how they may have co-evolved with particular life-history traits form our next scientific objective.

CONFLICT OF INTERESTS
The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Steve Smith and François Criscuolo conceived the study. Steve Smith, François Criscuolo, Franz Hoelzl and Sandrine Zahn wrote the text. All authors commented on drafts of the manuscript.

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