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REVIEW

Evolutionary ecology of telomeres: a review

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Telomere-induced selection could take place if telomere-associated disease risk shortens reproductive life span and differently reduces relative fitness among individuals. Some of these diseases first appear before reproductive senescence and could thus influence ongoing selection. We ask whether we can estimate the components of the breeder's equation for telomeres, in which the response to selection (R, by definition "evolution") is the product of ongoing selection (S) and heritability (h^2). However, telomere inheritance is a conundrum: in quantitative genetics, traits can usually be allocated to categories with relatively high or low heritability, depending on their association with relative fitness. Telomere traits, however, show wide variation in heritability from zero to one, across taxa, gender, ethnicity, age, and disease status. In spite of this, there is divergence in telomere length among populations, supporting past and ongoing telomere evolution. Rates of telomere attrition and elongation vary among taxa with some, but not complete, taxonomic coherence. For example, telomerase is commonly referred to as "restricted to the germ line in mammals," but inbred mice and beavers have telomerase upregulation in somatic tissue, as do many ectotherms. These observations provoke a simplistic understanding of telomere evolutionary biology—clearly much is yet to be discovered.

Keywords: telomeres; life history; aging; longevity; selection; heritability; mechanisms

Introduction

Telomeres comprise repeated sequences of noncoding DNA in association with proteins of the shelterin complex (Fig. 1). They protect the ends of chromosomes from various insults, such as reactive oxygen species (ROS) and other free radicals, but they also shield coding DNA from the DNA-damage response pathways.[1,2] In many organisms, the repeated telomere sequence is gradually lost with age, owing to the end replication problem and assault from free radicals.[3,4] However, telomere length may be maintained or restored by telomerase,[5] a reverse transcriptase coupled to an RNA template that replaces the telomeric sequence TTAGGG/CCCTAA in all vertebrates.[6] Rarely, telomeres are also renovated by homologous recombination and copy switching (alternative lengthening of telomeres[7]). All telomere loss at replication, however, cannot be compensated for, and, at a critical stage of telomere shortening, the cell enters a senescent stage and cell division ceases (replicative senescence[6]). Among species, the rate of telomere shortening is correlated with life span,[8,9] which has generated considerable attention from evolutionary biologists, since telomere dynamics may affect the aging process per se (longevity) and have downstream effects on classic life history trade-offs, such as current versus future reproduction.[10] Therefore, mechanisms that regulate telomere dynamics may be subject to selection that varies with the ecology of organisms.

It is clear that there is a range of mechanisms involved in the processes that govern telomere dynamics. Across Metazoa, these vary with, for example, homeothermy versus ectothermy, regenerative ability of tissue, and variation in telomerase

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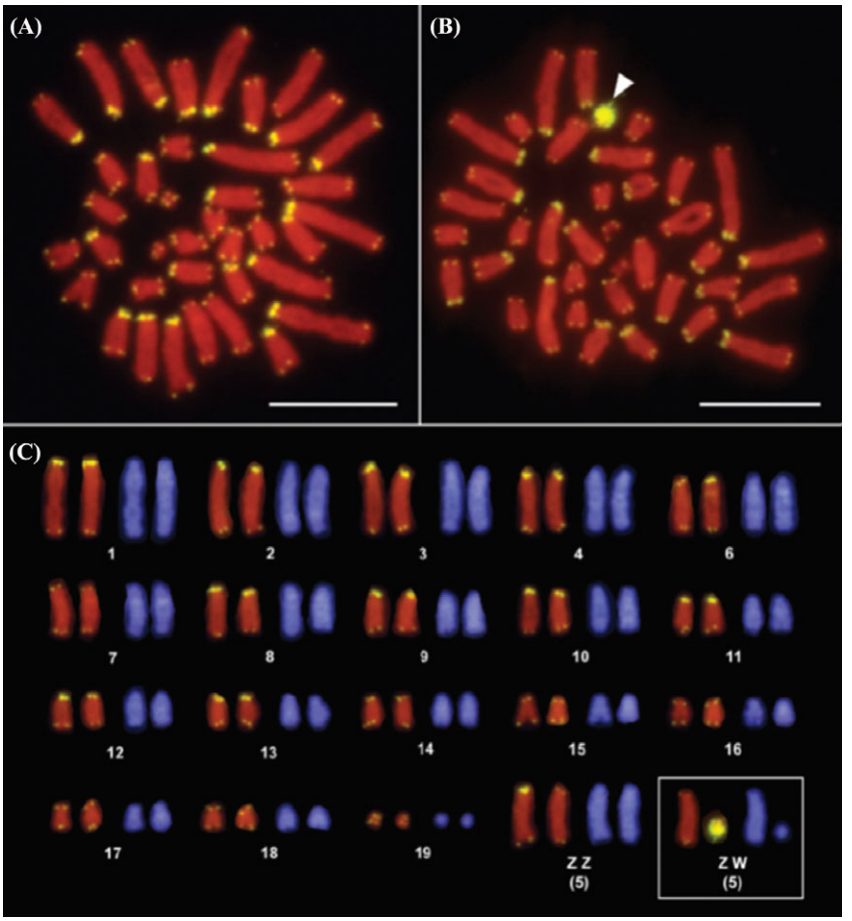

Figure 1. Chromosomal locations of the (TTAGGG)*n* repeated sequences in (A) male and (B) female *Lacerta agilis*. The arrowhead indicates the hybridization signals of the (TTAGGG)*n* sequence on the W chromosome. Scale bars represent 10 μm. (C) Full karyotype of *Lacerta agilis* with telomeres as in A and B. Micrographs from Ref. 176.

production across tissue and taxa.[11] Even within monophyletic taxonomic groups that share an ectothermic lifestyle, such as squamate reptiles (snakes and lizards), there are complex patterns of telomere dynamics,[12,13] with increases and decreases of telomere length in relation to different levels of telomerase production through life. These differences may be apparent even within the same species, such as between males and females[8,14] or between sexual and asexual individuals that may exhibit profound differences in telomere regulation. For example, in flatworms (*Schmidtea mediterranea*), asexual individuals maintain telomere length somatically during asexual reproduction (fission or regeneration after amputation), whereas sexual individuals achieve telomere elongation through sexual reproduction.[15] Thus, there appears to be an increasing acceptance and awareness that there is no single, universal pattern of telomere erosion and that this scenario has been very much simplified by studies focusing primarily on laboratory mammal models and humans with little or no telomerase production in somatic tissue.

In recent years, and particularly since 2009 when Drs. Blackburn, Greider, and Szostak won the Nobel Prize for telomere and telomerase research, there has been a monumental increase in the research output on the associations between telomeres and a broad range of biomedical health-related and other biological phenomena. These areas range from cancer and disease research,[16] oxidative stress biochemistry,[17] assisted reproductive technologies,[18] developmental biology,[19] epigenetic control of telomere length,[20] and in- and outbreeding genetics[21] to life history

evolution.[6] In many cases, these phenomena are interpreted to be causally linked to telomere dynamics. However, formally attributing cause requires manipulative experiments, which are lacking for both practical and ethical reasons in humans. Therefore, a critical first step is to determine whether telomeres are likely to have a causal role in evolutionary processes; in other words, do telomeres act as a selection pressure per se with evolutionary repercussions?

Evolutionary biologists have primarily been concerned with the potential for telomeres to act as regulating mechanisms in the aging process. In particular, do telomeres or telomere dynamics exert selection on or mediate trade-offs, such as whether to invest now or later, depending on the risk of future telomere-related mortality? However, fitness costs may also arise from a number of telomere phenomena that are not related to aging per se. Examples of these include genetic diseases caused by particularly short or interstitial telomeres;[22] predation attempts early in life (perhaps stress and ROS-related psychosocially induced telomere erosion[23,24]); epigenetic, transgenerational moderation of parental ability to withstand base pair erosion, and discordant selection generated by different health issues, such as cancer and atherosclerosis.[25] Clearly, these factors may be linked and are not mutually exclusive.

In this review, we look beyond aging as the only role telomeres play in evolutionary biology. This necessarily involves nonmodel organisms in natural populations and how factors other than age affect and potentially bias telomeric effects on fitness. We briefly address key issues with methods of telomere measurement. We explore the correlations between telomeres and fitness and assess the evidence for their causal role in affecting fitness. We discuss telomere regulation as a source of targets for selection and take a quantitative genetics perspective, along with nongenetic effects, to establish a foundation on which to build a theoretical structure to understand fitness effects of telomeres. Many of the reviewed papers would fit into more than one of the categories below and, hence, to save space, we have tried to minimize multiple referencing of the same literature.

Thus, our review has the following structure: (1) the importance of telomere measurement; (2) telomeres as biomarkers and correlates of fitness components; (3) are telomeres causally related to relative fitness via disease or in themselves; (4) telomere regulation and dynamics: telomerase and other mechanisms; (5) quantitative genetics, sex chromosomes, heritability, and evolvability of telomeres; (6) and transgenetic and epigenetic effects on telomere traits.

The importance of telomere measurement

The choice of molecular techniques for measuring telomere length and attrition all have their respective pros and cons. This is not the right forum for an extensive debate on methods (see reviews in Refs. 26–30), but we highlight some of the controversy in the field.

The most common techniques used to measure telomeres in the literature are telomere restriction fragment (TRF) Southern blotting, quantitative PCR (qPCR), fluorescence *in situ* hybridization (FISH) ("chromosome painting" with a molecular marker), Flow-FISH, and single-telomere length analysis (STELA). For a comprehensive description of these methods, see Nussey *et al.*[30] and Montpetit *et al.*[31] TRF analysis has shown relatively low interassay variation but underestimates short telomeres. That said, DNA shearing may inflate the abundance of short telomere fragments, which hence may be relatively less of a problem in the TRF Southern blotting procedure. Such compromise of sample quality should also become an increasing problem if samples are compared through time and DNA quality is not controlled for or modeled (e.g., by nanodropping)[32] Most studies discussed in this review do not specifically analyze DNA quality (or at least do not report on such scrutiny). The TRF gel DNA smear can be analyzed with densitometry (such as ImageJ), in which smear "grayness" (i.e., fragment density) along a molecular size ladder can be used to estimate telomere size. These can then be further analyzed in a size distribution partitioned into different percentiles at the researchers' preference. Quantitative PCR techniques show more interassay variation (largely dependent on the pipetting skills of the researcher) but are relatively fast and inexpensive (and measure telomere volume rather than base pair telomere length directly, as TRF does). Flow-FISH, which "paints" telomeres that are measured using flow cytometry, has up to 17% interassay coefficient of variation[29] but, like qPCR, better identifies short telomeres than TRF. FISH can be used to

identify telomeres and their approximate extent *in situ* on chromosomes.[22] Finally, STELA has the highest performance in terms of detecting short telomere fragments but is time consuming and expensive.[27,30]

Results reported using different methods have sometimes agreed in the literature and sometimes disagreed. For example, a comprehensive meta-analysis of 40 studies comprising 36,230 participants, discussing a range of mechanisms, showed that women had longer telomeres than men, but only when using TRF Southern blotting methods and not in studies using qPCR or Flow-FISH.[33] Importantly, it has been suggested in previous work (e.g., Ref. 34) that low accuracy in qPCR makes it more difficult to detect differences between groups (such as gender differences), supported by citing extreme values in the literature (e.g., 2.3–28%).[34] However, in Gardner *et al.*'s[33] meta-analysis, the coefficients of variation for TRF Southern blotting were 1.4–12% for TRF Southern blotting and 1.7–11.1% for qPCR. Furthermore, much of the extreme differences in telomere lengths between the sexes (e.g., in sign, larger in men than women) can be attributed to real-time PCR work by a single research group (for details, see Ref. 33), in spite of running careful cross-checks of potential sources of error (such as coding errors, and with interassay coefficients of variation always below 6%). In summary, the methods used to estimate telomere length can have important implications for interpretation, so throughout this review we explicitly state the methods used for most cited work.

One issue that has, in part, dictated the choice of telomere assay method is the existence of interstitial telomeres, which seem particularly prevalent in birds.[35] These are remnants from previous chromosomal fusions and may act as recombination hotspots and contribute to genetic disease risk.[22,173] On the other hand, broken chromosomal ends can be stabilized by ribosomal repeats colocalized with the telomeric sequence,[36,37] and telomeres may have the additional effect of silencing genes located nearby (telomere position effects[38]). Furthermore, interstitial telomeres are included in estimates, such as qPCR, and it has been argued that they should be excluded from telomere analyses, especially in studies of aging processes, in which attrition of terminal telomeres may be particularly important if terminal telomeres serve as molecular clocks or counters of cell divisions. In general, the more interstitial telomere DNA there is, and the blunter the method, the more difficult it would be to obtain an accurate terminal telomere estimate (if that is what is desired). However, two comments are important in this context: (1) when telomere attrition is the target analysis, any method that assays total telomere content is adequate, under the assumption that interstitial telomere content can be considered constant between episodes of measurements (which is probably nearly always a valid approximation); and (2) many telomere analyses serve (or are likely to serve in the future) as part of selection analyses involving disease ecology and host–pathogen evolution. In this context, interstitial telomeres may prove as or more important than terminal telomeres, given their importance in genetic disease and chromosomal aberrations.[22]

Telomeres as biomarkers of aging and correlates of fitness components

Establishment of a causal relationship between telomere traits and components of fitness, such as longevity, should ideally involve manipulation of telomeres and assessment of manipulation consequences on the traits of interest. Few studies have managed to manipulate telomere traits (see below), but there is a rich literature that establishes correlative links between telomeres and components of fitness. One such study is that of Bakaysa *et al.* (using TRF)[39] on elderly Swedish human twins, which showed that the twin with shorter telomeres had three times the risk of mortality as their twin sibling with longer telomeres. Bakaysa *et al.* suggest that this effect is perhaps pleiotropic or environmental. Other research has had quite a different outcome. Bischoff *et al.* (using TRF)[40] analyzed the effects of telomere length on mortality risk and life span in people 73–101 years old. In this study, telomere length was a significant predictor of future life span only when age was not controlled for, in contrast to a similar study by Cawthon *et al.* (using qPCR),[41] who verified a positive relationship between telomere length and longevity, also with age held constant. Work using both TRF and STELA showed that exceptionally old people had an overrepresentation of ultra-short telomeres and that women had longer telomeres than men, at least partly explained by fewer ultra-short telomeres in women.[42] In research by Benetos *et al.* (using TRF),[43] telomere length is

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argued to "significantly contribute" to higher heart pulse pressure and pulse wave velocity in men but not in women, but, then again, causality is assumed rather than verified. Barrett *et al.* (using qPCR)[44] showed, in Seychelles warblers (*Acrocephalus sechellensis*), that telomeres shorten with increasing age and body mass and that shorter telomeres, and telomeres shortening faster, are predictors of mortality. Earlier work by Haussmann *et al.* (using TRF)[45] on tree swallows (*Tachycineta bicolor*) also convincingly demonstrates that tree swallows with long telomeres at 1 year of age have higher probability of survival for at least 3 years following the measurement.

Pauliny *et al.* (using TRF)[46] showed effects of longer telomeres on increased life span in sand martins (*Riparia riparia*). This work has since been criticized (e.g., Salomons *et al.*[47]) for using the Telometric program[48] to analyze the gel data, which, due to interpolation of data at high molecular weights, results in longer telomeres compared with densitometry-based analyses, such as ImageJ. However, Olsson *et al.*[49] did a complete selection analysis with both Telometric- and ImageJ-derived data on molecularly assigned lifetime reproductive success in sand lizards (*Lacerta agilis*). The analyses qualitatively showed very strong concordance between the techniques, and the results were only separated by a scaling factor. Thus, Pauliny *et al.*'s work seems robust and was the first to analyze a wild population with respect to two important fitness components, longevity and lifetime reproductive success (based on molecularly assigned parentage), the latter being an estimate of "true" relative fitness. Importantly, however, all these studies use cross-sectional data, with known age entered into the analyses, but this may still result in positive correlations between age and telomere length, either because individuals with short telomeres die young or because telomeres elongate with age. Thus, these data should be further corroborated by longitudinal studies in which telomere length along a temporal axis is analyzed with respect to fitness effects.

In human twins, the relative difference in telomere length remains similar through life,[50] and, across homeothermic species, the greatest telomere shortening seems to happen early in life.[11] This may generate among-individual differences in telomere length if there is considerable variation in the rate of telomere attrition and replenishment. Thus, DNA for telomere analyses in mammals predominantly comes from white blood cells, with a life span and exposure time to free radicals of 5–21 days, whereas for birds and ectotherms it comes from nucleated red blood cells, with an approximate life span of 120 days in humans[51] and 800 days in reptiles (http://www.seaturtle.org/pdf/StacyNI_2011_ClinLabMed.pdf). Differences in age or place of cell origin could thus affect telomere length and have not been investigated in nonmodel organisms (red blood cells are formed in the bone marrow and white blood cells in the bone marrow, lymph nodes, thymus, and spleen).

In a captive population of zebra finches (*Taeniopygia guttata*), telomere length at 25 days was the best predictor of life span, although individuals that lived the longest (some for 11 years) had the longest telomeres at all time points through life. Interestingly, reproduction further shortened telomeres, suggesting a classic trade-off between longevity (somatic maintenance) and reproductive investments (using qPCR).[52] Olsson (using qPCR)[53] reported similar effects in wild sand lizards (*L. agilis*), with faster embryonic growth predicting shorter telomeres at hatching and longer telomeres in hatching offspring than those that died in incubation and longer telomeres in recruiting young than those that died as juveniles. In a wild bird population (barn swallows, *Hirundo rustica*), telomere length at hatching was not a significant predictor of life span (using qPCR).[54]

Simon Verhulst's team used two sampling events to estimate slopes of telomere change for the different percentiles in their telomere distributions and discovered that this slope was steeper (i.e., greater telomere base pair loss) in higher percentiles (longer telomeres) than lower ones. They then examined whether longer telomeres that lost more base pairs were also the best predictors of survival and (seasonal) reproductive success.[55] Indeed, this was the case: individuals that lost more base pairs were more likely to disappear ("die"), and this effect was stronger in individuals with longer rather than shorter telomeres. Experimental work using targeted transposition of repeat retrotransposons to try to maintain chromosome length failed to demonstrate a relationship between telomere length and life span in laboratory-kept fruit flies (*Drosophila melanogaster*) but demonstrated a negative relationship between telomere length and fecundity, fertility,

and embryo development.[56] Embryos with longer telomeres showed higher hatching failure.

Telomere length and telomerase, and their relationships to longevity, have yielded mixed results in comparisons across taxa. Lorenzini *et al.*[57] made an attempt at comparing the relationships between DNA double-strand break (DSB) recognition, versus telomere length, and longevity across 15 species of mammals, while controlling for phylogenetic relationships. DSB recognition was significantly (linearly) correlated with species longevity ($r = 0.903$, $P < 0.0001$, $n = 12$). When the authors performed the corresponding analysis for telomere length, they concluded that the relationship was not significant. However, data were missing for two species, and this relationship was significant at α set to 0.05 ($r = -0.062$, $P = 0.0547$, $n = 10$; with body mass partialled out, $P = 0.222$). With limited sample sizes, the conclusions are clearly labile. Furthermore, work by Gorbunova *et al.*[58,59] demonstrates that telomerase activity has coevolved with body mass and not with life span.

One of the most thorough and comprehensive studies on the relationships between short telomeres and aging was conducted on zebrafish (*Danio rerio*) in multiple tissues (using qPCR).[19,60] Contrary to expectations, telomeres shortened to a critical length only in specific tissues, independently of their proliferation rate. Notably, short telomeres accumulated at the same rate in the highly proliferative gut tissue as in muscle tissue that is far less proliferative. In both tissue types, telomere shortening anticipates age-associated disease, including cancer.[60] Interestingly, another research group found that telomerase production in zebrafish is lifelong in all somatic tissue and that telomere length was maintained through life in all tissue and was unaffected by fin clipping–enforced regeneration.[61]

Among studies, there have been considerable differences in exploring the relationships between telomere traits and survivorship or life span, with some confirming and others dismissing positive telomere–fitness relationships. One study on tropical pythons (*Liasis fuscus*) refutes such a relationship (using TRF Southern blotting).[13] In this study, telomere length increased early in life but then asymptotically leveled out in older age,[13] resulting in longer telomeres in females than males. No relationship with survivorship was found.[13] A second study by Ujvari *et al.*[12] on frill-neck lizards (*Chlamydosaurus kingi*; using qPCR) showed short telomeres in young lizards followed by a telomere growth period in mid-life and attrition in older lizards. Telomere length variance was maintained in older animals, with an overall curvilinear telomere distribution (Fig. 2)[12]

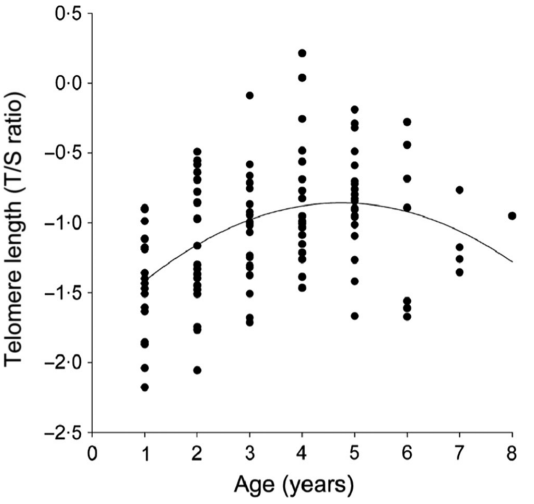

Figure 2. Cross-sectional relationship between $\log_e$-transformed telomere length (T/S ratio) and lizard age (Reprinted from Ref. 12).

Ujvari *et al.*[12] discuss age-specific variance in published telomere data and, potentially, what such data reflect in terms of past and ongoing selection. The telomere range in tropical pythons was ~17.2–32.5 kb,[13] with a clear tapering off in variance with age. The telomere length range was ~9.4–23.1 and 4.4–23.1 kb, respectively, for sand martins and dunlins.[46] For sand lizards, the corresponding ranges were 14.8 ± 1.4 kb (SD) in a Telometric analysis and 10.6 ± 1.5 kb in an ImageJ analysis of the entire lane length.[49] Thus, telomere length varies among species, and covariation between mean length and variance is probably statistically expected (as for much phenotypic data). Since variance components also include variation due to error of measurements, reporting of inter- and intraassay coefficients of variation is fundamental. On that note, two observations are relevant in terms of choice of methods: (1) an increased variance due to methodology in telomere traits (e.g., wider variance in qPCR than in STELA) makes the detection of significant covariation between telomere attributes and other parameters more difficult to detect, that is, the test becomes conservative; and

(2) the test is not more likely to be biased or cause spurious correlative patterns unless there is significant covariation between increasing errors of measurement simultaneously in both traits,[62] which is not expected and can be tested for.[62]

Another potential telomere–fitness correlation appears at the level of reproductive effort in life history ecology.[10] An intuitive prediction is that more effort should result in shorter telomeres. However, in humans, the pattern seems to be the exact opposite (although of borderline significance: $P = 0.045$), with the authors suggesting that lower telomere attrition with more children stems from protective effects of cooperative breeding with more support from "grandmothers."[63] In fish, (Atlantic silversides, *Menidia menidia*), more fecund individuals had both shorter life span and shorter telomeres (using qPCR).[64]

A study by Bauch *et al.*[55] claimed that telomeres are biomarkers of fitness components (e.g., correlates of arrival date at breeding grounds and reproductive success) rather than being causal, dynamic regulators of fitness. Their study of the tern (*Sterna hirundo*) showed that longer telomeres are "better" (authors' definition) indicators of aging, because longer telomeres correlate with fitness parameters, whereas shorter telomeres that lose fewer base pairs do not. This is in contrast to their own work from 2 years earlier, which demonstrated that terns with short telomeres had higher reproductive output;[65] both of these studies were on bird class II telomeres.[55] A third paper from the same group, this time on jack-daws (*Corvus monedula*), demonstrated shorter life span in birds with shorter telomeres. However, this time, the team worked on class I telomeres (i.e., the shortest bird telomere class[35,47]), so perhaps this is part of the explanation for these differences in outcome.

Another form of reproductive investment is sexually selected color traits. If high-level ornamentation comes at a maintenance cost (which seems reasonable), telomere length and attrition may reflect aspects of the aging process[66] if they correlate with exterior, phenotypic traits that act like "health certificates" to partners and rivals. Giraudeau *et al.*[67] investigated the links between color fading during the mating season in Australian painted dragon lizards (*Ctenophorus pictus*) and telomere attrition. They demonstrated that levels of ROS at the onset of the mating season were unrelated to initial telomere length, but lizards that better maintained their coloration also lost more telomere bases (using qPCR).[67] Furthermore, work on the same species used its polymorphism with differences among morphs in head color and associated reproductive behaviors, including level of aggression and investment in reproduction. In accordance with predictions, morphs with high investment in reproduction also had shorter telomeres, which captures the relatively lower levels of somatic maintenance (using qPCR; Fig. 3).[68]

The underlying reasons for sexual differences in telomere length have been a contentious issue[69] and are still fiercely debated.[8] However, one thing is clear: when two sexes differ in key traits, such as reproductive investment, heterogamety, aging trajectories, sexual dimorphism, and disease susceptibility, the prediction is that telomere dynamics should differ between the sexes; we expect differences among sexes depending on their current and past evolutionary biology. Studies on nonhuman taxa have both confirmed and dismissed sex effects on telomere length, attrition, and ongoing selection. Barrett and Richardson[8] detailed sex differences among the taxa insects, "reptiles" (polyphyletic), aves, and mammalia, and, without taking phylogenetic dependence into account, 10 species show shorter female telomeres, 10 show no difference, and, in 29 species, males have shorter telomeres. Barrett and Richardson[8] also provide an insightful discussion of potential genetic mechanisms explaining among-taxa differences in telomere dynamics (such as the role of heterogamety), but data are (in our opinion) too meager to provide a general theory. Interestingly, in the marsupial Tasmanian devil (*Sarcophilus harrisii*), telomeres are strikingly dimorphic between homologues, with a lengthening process during spermatogenesis (on the Y chromosome) and a shortening process during oogenesis.[70] Furthermore, the Tasmanian devil is fraught with one of the few described contagious cancers in wild populations, and the tumors resulting from transfer of the malignant cells during agonistic interactions all have characteristically short telomeres.[70]

Few species have been examined in which males' and females' life history strategies and associated reproductive investments are drastically different. One such case, however, is the red-sided garter snake (*Thamnophis sirtalis parietalis*),[14] in which males and females differ in their corticosterone

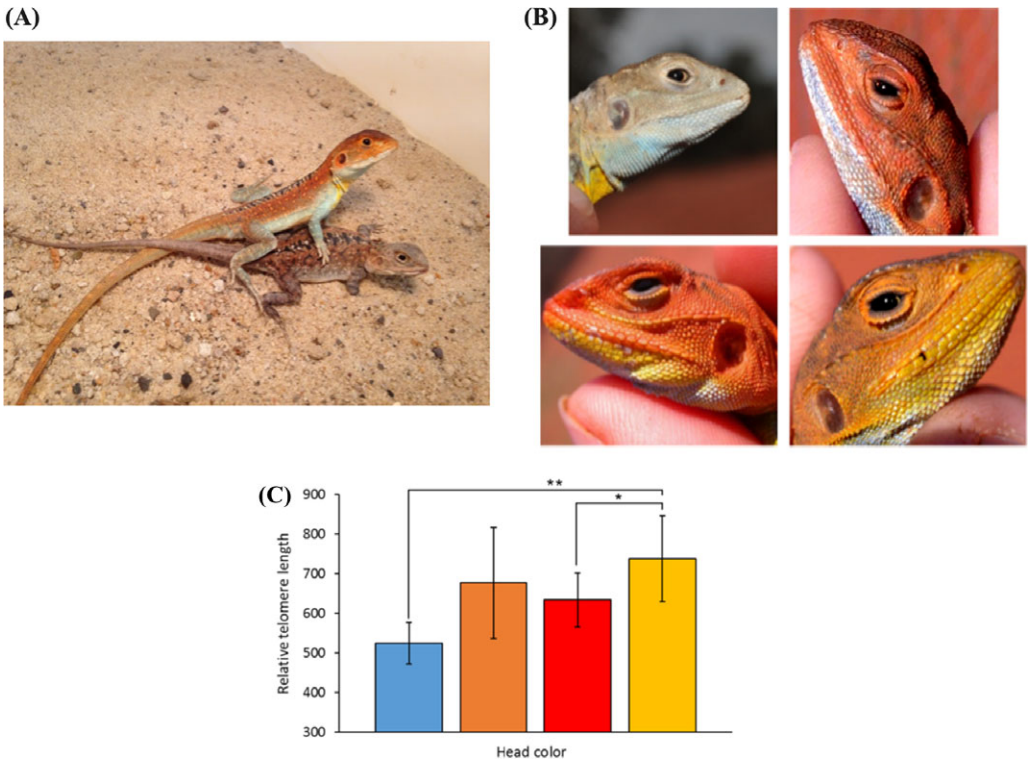

Figure 3. (A) Male and female painted dragon (*Ctenophorus pictus*). (B) The four male morphs of painted dragons. (C) Mean (± SE) relative telomere lengths (RTLs) of the four male morphs. RTL of yellow males was significantly higher than red and blue males (B and C from Ref. 14; see text) (© CRF).

levels (higher in males) following hibernation and males engage in mate searching, courtship, and mating behavior, whereas females have a more passive role. We predicted that telomere erosion would be more pronounced in males than females for these reasons, and this indeed proved to be correct; males have a (negative) quadratic telomere decline with age, whereas females maintain their telomeres without noticeable attrition (Fig. 4A–D).[14] Thus, identical methods used for both sexes (qPCR) by the same person (Nicky Rollings) strongly suggest that these results are robust. It is also interesting from a natural history perspective that these female garter snakes reproduce every other year, whereas males engage in this energetically costly courtship yearly. Furthermore, male size does not seem to affect male mating success (and therefore age, as snakes have indeterminate growth). This may generate very different selection scenarios for males and females favoring longevity and somatic maintenance in females, as suggested by their lack of telomere attrition.[14] Although they did not find sex-specific effects, a similar argument, based on enhanced reproductive output in older animals, has been evoked to explain cross-sectional patterns of telomere length increases with age in edible dormouse (*Glis glis*; using qPCR). The telomere dynamics were characterized by decreases in telomere length from early to middle age and then increasing telomere length throughout old age. This age-specific pattern in cross-sectional data sets can be explained by differential selection at earlier life stages. However, Hoelzl *et al.*[174] also conducted a laboratory-based study using repeated measures (longitudinal data) to verify that relative telomere length decreased in subadults (<5 years of age) but increased in older individuals (5–10 years of age). These longitudinal results mirror those found in a previous, field-based study in dormice by Turbill *et al.* (using qPCR),[128] who found age-specific telomere lengthening in adults but the opposite trend in subadults during the spring–autumn active period.

Given that telomeres shorten with each cell division in many species, it is not surprising to find

Figure 4. Sex differences in age-dependent telomere length, with no relation in (A) females but a curvilinear decline of telomere length with age in (B) males in the red-sided garter snake (*Thamnophis sirtalis parietalis*). Females show a much less active reproductive strategy. (C) A passive female (the large, central head) is surrounded by smaller heads of energetically, courting males. This mating behavior typically takes place in (D) large competitive aggregations emerging from hibernation in limestone sick holes in spring (© CRF, from Ref. 68).

correlations with growth, body size, and sexual size dimorphism and sex-specific telomere length. Indeed, this is an argument for sex differences in telomere length in species in which females have longer telomeres than males (when males are the larger sex).[69] Ongoing selection on body size and corresponding selection in the wild on telomere length have been demonstrated in a (correlative) quantitative genetics field study of sand lizards (using TRF).[71] Similarly, artificial selection for shortened tarsus length (a measure of body size) in house sparrows (*Passer domesticus*) resulted in a correlated shortening of telomeres (using qPCR).[72]

The mechanistic relationship between body size and telomere length and their shortening with successive cell divisions suggests that large taxa, which require more cell divisions to reach mature body size, should have either shorter telomeres or increased telomerase activity, which should also lead to an increased cancer incidence; a prediction for which there is no evidence (Peto's paradox).[73] Work using zebrafish telomerase knockdown mutants shows an increase in short telomeres coinciding with growth arrest, apoptosis, and other aging symptoms, such as spinal curvature, liver and retina deterioration, and infertility.[74] Telomerase-dependent rescue of short telomeres by elongation with TA-65 (see methods below), followed by lower levels of DNA damage, has been demonstrated to increase health span in mice with effects on glucose tolerance, osteoporosis, and skin fitness without significantly increasing global cancer incidence. Nevertheless, telomerase activity correlates with body mass in rodents (repressed in larger species) but not with life span, while controlling for phylogenetic relationships.[75,76] This contrasts with the work by Haussmann *et al.*,[77] who demonstrated a slower loss of telomere bases in long-lived than short-lived birds and argued for such a relationship in mammals in the literature.[6,78] Later work by Dantzer and Fletcher,[9] however, lends further support to the hypothesis that slow-aging wild animals lose telomeres more slowly than fast-aging ones (across Aves, Bivalvia, Reptilia, and Actinopterygii).

Ideally, growth should be manipulated while leaving other systemic parameters, such as body temperature, alone, but very few systems allow for such experimentation. A clever way around these problems was Pauliny *et al.*'s[79] manipulation of growth hormone (GH) levels in salmonids by

comparing transgenic fish (with doubled-up GH genes). This resulted in extreme growth rates compared with wild-type fish in a split-brood design using the same females for both treatment and control. As predicted, GH-manipulated fish suffered much higher telomere erosion (using qPCR).[79] The same research group looked for effects of compensatory growth on telomere length in brown trout,[80] but in this experiment the spurt in growth activity generated by increased GH transcription (not measured) did not affect telomere shortening (using qPCR).

Growth in ectotherms is the outcome of synergy between a complex set of drivers, such as temperature and innate capacity for growth set by acclimation processes.[81] Rollings *et al.*[82] explored catch-up growth effects in mosquito fish (*Gambusia holbrookii*) and found a weak difference among treatment groups, with fish at constant 20 °C having shorter telomeres (using qPCR) than fish in treatments experiencing a gradual change from 30° to 20 °C. In (homeotherm) rats, maternal undernutrition can influence growth and longevity in male offspring,[83] with growth retardation during fetal life followed by postnatal catch-up growth being associated with a shorter life and shorter telomeres. This relationship may be a mechanistic link between early low growth rate and degenerative disease.[84]

Among-individual variation in telomere length may differ 100-fold between species in the same class, and some of this variation has been suggested to be due to differences in shortening rate linked to life span; mice, for example, have faster telomere shortening rates than humans and a shorter life span.[85] However, Vera *et al.*[85] showed with Kaplan–Meyer survival analyses that percentage of short telomeres (measured using HT-QFISH) at the end of each month was a better predictor of life span than telomere attrition rate.[85] In nestling barn swallows, however, telomere attrition rate (using TRF) was not found to predict life span.[54] However, in this study, less than 5% of the offspring were recruited locally, and 95% were assumed dead within a year. Thus, the population on which selection acted on posthatching was 1180 individuals, which would have been the correct sampling cohort; to expect telomere effects to influence longevity when 95% of the population is already gone as a result of multifactorial selection, including that due to telomeres, is indeed to have faith.

Given that nonexperimental telomere versus phenotype trait relationships are correlations, either may be assigned as the response or predictor variable; thus, when telomere traits are assigned as the response variable, researchers have assumed telomere erosion as a cost of some investment, such as fast growth or reproduction. Conversely, when the telomeric trait is assigned as the predictor, the researcher assumes that telomere maintenance is costly and is reflected in the response of some life history trait. Alternatively, the causal arrow may rotate in a dynamic or positive feedback relationship. The true relationship is at the mercy of the interpreter or other supporting data. Parolini *et al.* (using qPCR)[86] took the first approach and assumed that telomere shortening leads to cell senescence with negative consequences for organismic function in barn swallows (*H. rustica*). Telomere length declined drastically during the maximal, early nestling growth period, but tail and wing length increased with relative telomere length, more so in males than females.[86] Likewise, in brown trout (*Salmo trutta*), Adriaenssens *et al.* (using TRF)[87] showed a relationship between telomere length and individual aggression and exploration, assuming that a shorter life span, as a result of shorter telomeres, selects for a bolder, more aggressive personality (linked to the pace of life syndrome).

Are telomeres causally related to relative fitness via disease or in themselves?

Research on telomere evolution has long been fraught with a dilemma: how to independently manipulate the telomere traits themselves (e.g., length and attrition) independent of other physiology or fitness components. For example, if telomeres were eroded using ROS[17] or rescued from those using antioxidants,[88] effects on other life history parameters or cell–cell signaling are likely to compromise the experimental outcomes and interpretations. Thus, there has been little room for experimenting with the trait itself and recording a response in fitness or a component thereof (such as longevity).

Simons[89] published a comprehensive review in which he compiled research outcomes from the mechanistic literature, in particular from knockout and overexpression studies on aging, a major advance in the right direction. His review sought to identify costs and benefits of telomere traits on a

component of fitness and test whether there is a critical telomere length at which senescence is induced. If so, variance in telomere length should decrease with age (like in a funnel diagram), as predicted by theory. Simons found no such effect in a large meta-analysis comprising 16,384 human subjects, and neither did he find many effects suggesting that telomeres causally affect aging in laboratory models (e.g., mice, *Arabidopsis*, *Drosophila*). These results were independent of the methods used in the molecular analyses (the same trends for qPCR and TRF).

An example of potential threshold effects of telomere lengths on cell viability are Ujvari *et al.*'s[12] studies of telomere biology in frill-neck lizards (using qPCR), in which they conclude, "telomere length dynamics reflect an adaptation to maintain telomere length above a critical minimum in order to maintain cellular homeostasis." Although telomeric signaling of a critical minimum and cell-suicidal apoptosis are potential outcomes of this process, a suite of work reviewed here confirms covariation between telomere length and attrition and variation in components of fitness. Life history traits and disease risk, as continuous variables, covary with telomere length and attrition dynamics, which is not predicted by a critical, minimal telomere length effect. Thus, Ujvari *et al.*[12] seem to conclude that telomere length above a critical minimum is irrelevant—noise from lag phases in telomere length-attrition and telomerase activity—and neutral to selection (our interpretation); however, the critical minimum idea fails to explain broad, quantitative patterns in telomere associations in evolutionary ecology and genetics.

Gerontological and epidemiological arguments in the perspective of evolution of life span naturally merge, since older people also suffer more from poor health.[90] But how strong is the evidence that telomeres play a causal role in disease and therefore act as selection pressures in current, ongoing evolution in natural populations? Simons[89] suggests that, when epidemiological associations are "very strong, and are corroborated by experimental evidence from animals or other supportive evidence, such as in the relationship between smoking and lung cancer . . . , causality can be deduced." To assess this, we need insight into fundamental telomere properties related to "disease," here defined as any compromise of the fitness of a phenotype.[91,92]

From the perspective of evolutionary biology, disease constitutes just another selection pressure.[93] Most importantly, we are interested in processes that act before reproductive senescence. Longevity with no added bonus to fitness is evolutionarily irrelevant. What is important is telomere properties that have effects while an organism is reproductive but before the phenotype goes undetected by selection in the postreproductive years (with the qualification that, in the rare cases of populations with grandparental care, "grandparents" may contribute to fitness). Telomeres occur in all metazoans, and there should be parsimonious explanations for telomere dynamics, such as what role(s) conserved genetic mechanisms play in aging and life history biology at large. Thus, explanations for telomere causality in aging and life span evolution should not be unique to humans.

Evolutionary explanations in human pathology often led to the strong, repeated claim that telomere length is causally related to ". . . the cancer–atherosclerosis trade-off across humans." Aviv and colleagues suggested that "this trade-off has been principally established through the force of evolution . . . "[25,94] Long telomeres are associated with risk of melanoma, whereas short telomeres are associated with atherosclerosis. However, to what extent these arguments apply to other disease is open to debate. Work by Strefford *et al.*[95] shows that short telomeres are significant predictors of leukemia incidence, but no explicit process has yet been identified that selects against very long telomeres (although theoretically it could also be driven by atherosclerosis in this case). Links between early- and late-life telomere length and association with cancer risk were investigated in a large sample by Nordfjäll *et al.* (959 human cases).[96] They concluded that early short telomeres were not predictive of late-life malignancies. However, telomeres are associated with other disease states. For example, chromosome rearrangements resulting from telomere loss and those found in cancer cells implicate telomere loss as an important mechanism for the chromosome instability contributing to tumor formation.[97] Typically, chromosome ends without sufficient telomere repeats are prone to fusion, and the resulting dicentric chromosomes compromise the ability of cells to continue cell division. In telomerase gene null mutants, for example, chromosome fusions and other molecular

changes in later generations result in a wide range of phenotypes, including failure to reproduce, poor growth, and accelerated rates of aging.[91] Research on pancreatic carcinoma and osteosarcoma is consistent with telomere shortening, and more than 10% of chromosome ends completely lack telomeric signals. This is indicative of extensive genomic instability (using FISH analyses).[98] In a similar study on breast carcinoma, a PCR-based method (telomere-associated repeats) revealed that malignant breast lesions (tumors) contained telomere fusions, unlike tissue from healthy volunteers.[99]

Other more immediate links between telomere molecular genetics and disease include specific mutations in the telomerase (*TERT* and *TERC*) components,[100] resulting in short telomeres and a higher incidence of idiopathic pulmonary fibrosis (IPF);[101] IPF causes depletion of epithelial alveolar cells, senescence, and fibrosis. Telomeres also shortened with age almost twice as fast in patients with ulcerative colitis, a chronic inflammatory disease that predisposes to colorectal cancer.[102] Limiting the number of cellular divisions in human cells leads to a preneoplastic (i.e., potentially precancerous) growth arrest state referred to as *senescence*.[103,104] However, premalignant cells expressing viral oncoproteins can bypass senescence and move into *crisis* (or terminal telomere shortening). At this stage, telomeres are so short that end-to-end fusions occur, followed by bridge–breakage–fusion cycles. This stage is only rarely bypassed by human cells,[103,104] and it is currently unknown how common this is in other taxa and to what extent the phenomenon constitutes a selection pressure in natural populations.

Many chronic diseases are believed to have their roots in fetal development, in particular in maternal stress during pregnancy,[23,105,106] and hence such factors may well dictate selection in relation to fetal mechanisms and programming. Although these effects are clearly multifactorial, one factor at the cellular level is the adverse impact of the intrauterine environment on telomere dynamics.[105] This may be caused by psychosocial stress during pregnancy (as measured on the Holmes and Rahe stress scale), which is negatively associated with telomere length in human infants[23,105] (using qPCR and TRF, respectively) and associated with rapid upregulation of telomerase levels in association with cortisol.[107] This psychosocial effect, however, did not persist in terms of telomere attrition after age 30.

A critical question that arises from these studies is how telomeres in a relatively short-lived cell mechanistically influence life span in a much longer-lived phenotype (in other words, what is the relationship between replicative, cell-culture senescence and whole-organism, phenotypic senescence). One pathway through which telomere length at birth could affect components of fitness, such as life span, is through genomic instability (i.e., increased risk of malignant tumor formation). Moreno-Palomo *et al.*[108] assessed this with a two-pronged approach: (1) cord blood from human newborns was analyzed with respect to telomere length (using TRF) and basal genetic damage, and (2) cells of a subgroup were challenged for induced genetic instability using mitomycin C (a mutagen). In both cases, shorter telomeres were associated with greater levels of genetic damage (i.e., were more genetically unstable).[108] Since telomere length varies among individuals at hatching or birth, an obvious next question is whether selection can act via interaction between life history stages[109] so that a newborn/hatchling telomere trait is affected by selection on an adult trait via genetic correlations. This could causally affect probability of survival and ultimately life span. The best evidence is probably from experimental work on the offspring of transgenic nematodes (*Caenorhabditis elegans*), where nontransgenic worms in the F1 generation retained life span–extending effects from elongated telomeres.[110]

Studying the causal effects of telomeres on fitness in wild populations, especially when no such effects can be verified, is complicated by a number of factors. For example, in work with relatively small sample sizes, high variance in molecular methods, low recapture rates in the wild, or when only half of the fitness equation is covered (i.e., reproduction is overlooked), the lack of significant fitness effects on telomeres becomes a curtailed test at best, or misleading at worst. For example, in sand lizards, juvenile mortality is ~0.8–0.9 depending on year;[111] the minimum–maximum values for lifetime reproductive success in males are zero (overrepresented) to 76 offspring.[49] Thus, judging selection on telomere length on the basis of differential survival in already adult individuals, while ignoring juvenile mortality and sexual selection, would only capture a fraction of ongoing selection.

The most complete integration of proximate and ultimate evolutionary telomere biology is probably

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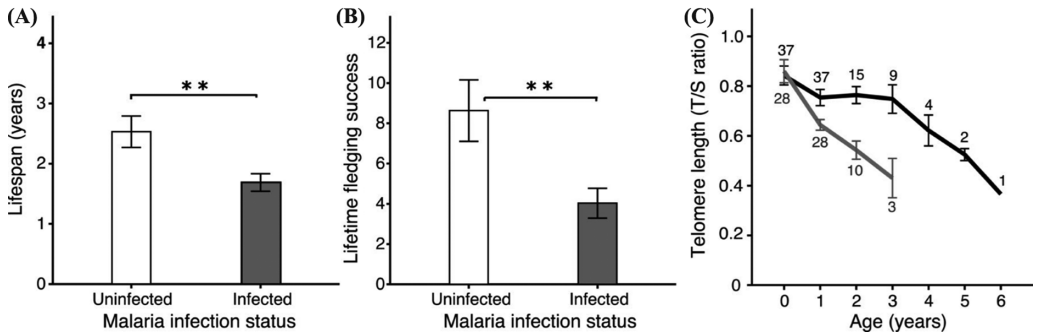

Figure 5. **Relationships between malaria infection status, life span, lifetime fledging success, and telomere length in wild great reed warblers (abbreviated interpretation from Ref. 112). (A) Uninfected birds lived longer than birds with chronic malaria infection. (B) Uninfected birds produced more lifetime fledglings than chronically infected birds. (C) Relationships between telomere length and age in uninfected (black line) and infected (gray line) great reed warblers (over life). Telomere length decreased with age but at a steeper rate in infected birds. Error bars represent mean ± SEM. *n* values are given for each age group (uninfected above and infected below error bars).**

the work on reed warblers (*Acrocephalus arundinaceus*) by Staffan Bensch and Dennis Hasselqvist's group at Lund University in Sweden. Their combination of field studies demonstrated long-term costs of malaria infection on life span and lifetime reproductive success and was further supported by an experiment in captivity demonstrating direct telomere degradation in infected birds. They conclude that chronic malaria infection causes a series of small adverse effects, of which telomere attrition (using qPCR) was one, eventually leading to phenotypic impairment (Fig. 5A–C).[112] Such telomere attrition occurred throughout six major tissue types in infected birds.[113] This important work leads the way for future studies that investigate aspects of disease ecology in telomere function and evolution.

An alternative approach going forward to look for telomere causality on components of fitness is through manipulative experiments. An example of this approach is recent work on zebra finches[114] in which "healthy aging" was examined after treatment with TA-65. TA-65 is a herbal supplement that is purported to function as a telomerase activator[115] and increase telomerase activity by upregulating telomerase transcription factors binding to the activation domain of the telomerase gene.[116] Reichert *et al.*[114] showed that, when telomeres were longer, regrowth of flight feather was faster in TA-65–treated individuals and, hence, longer telomeres were concluded to be indicators of overall health and viability. A similar experiment on ectothermic dragon lizards (*C. pictus*), using elevated TA-65 dosage to compensate for lower night metabolic rate, had no effect on telomere length (using qPCR).[68] Clearly, a method of reliably manipulating telomere length that has few pleiotropic effects would be a boon for those interested in elucidating the causality of telomeres in the context of evolutionary ecology.

Telomere regulation and dynamics: telomerase and other mechanisms

A number of different mechanisms affect telomere length through life. Differences in telomere length are indeed set at the earliest stages of cell life. Depending on telomere length in different hematopoietic stem cells, telomere length varies among chromosomes in blood cells.[91] An additional complexity is that telomere length on different chromosomes covaries with the length of the chromosomes themselves, with such variation conserved through life and partly inherited.[117] Very short and dysfunctional telomeres activate a DNA response pathway leading to a number of outcomes; DNA repair, cell senescence or apoptosis, and preferential lengthening of shorter telomeric tracts by telomerase.[1,6] TEL1, an ataxia–telangiectasia mutated family checkpoint kinase, seems to play an important role in such telomere elongation, as cells lacking TEL1 have short telomeres (measured using qPCR) and show reduced recruitment of telomerase components to telomeres.[118] These processes are also part of autoimmune or immune-mediated disease and pathophysiological processes.[119] Available data indicate that telomere and telomerase homeostasis is modified in systemic inflammatory

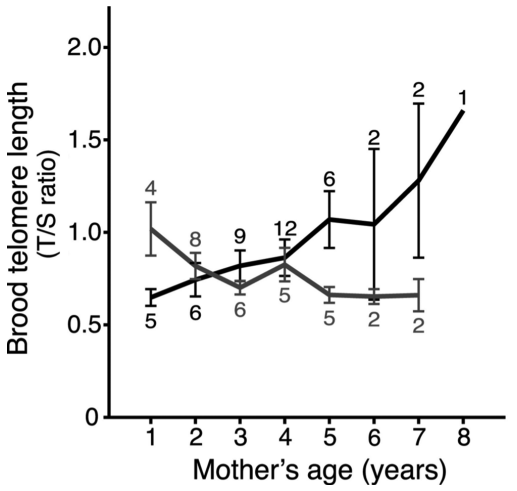

Figure 6. **Relationships between mother's age and the mean early-life telomere length of her offspring in relation to mother's malaria infection status in wild great reed warblers (abbreviated interpretation from original Ref. 112). Uninfected (black line) and malaria-infected (gray line) mothers differed in the relationship between the mother's age and the brood mean telomere length of her offspring.**

disease (e.g., lupus erythematosus, rheumatoid arthritis, and granulomatous disease) and contributes to premature immunosenescence.[119] Thus, this seems to be an avenue for selection to operate on telomere and telomerase properties.

Observations of telomere restoration have been made with nuclear-transfer techniques using cultured somatic cells (cloning) in sheep, without involving germline cells.[120] Shiels *et al.*[120] showed that telomeres (using TRF) were shorter in all three nuclear-transfer animals than in age-matched controls. However, in another cloning experiment in cattle,[121] cloned bovine fetuses had telomeres (also using TRF) that were extended in length beyond those in both newborns and age-matched controls.[121] Thus, Lanza's study shows repair of eroded telomeres during the cloning process, which was not the case in sheep.[120] The exact mechanistic difference(s) between these results is not fully understood.[121]

Another genetic factor that has been suggested to affect telomere length is inbreeding depression (e.g., with long telomeres and high somatic telomerase levels throughout the body[122–124]). For example, Bebbington *et al.*[21] analyzed molecular inbreeding data on Seychelles warblers (*A. sechellensis*) and showed that inbreeding negatively affects telomere length (using qPCR). In many taxa, it would be advisable to control for family effects in such analysis (since siblings are unlikely to be independent statistical units of inbreeding), but, in Seychelle warblers, clutch size equals one in most cases, so sibship is unlikely to be a confounder (David Richardson, personal communication). Olsson[53] reported near-identical approaches in sand lizards (*L. agilis*) and showed that inbreeding affected telomere length negatively when family effects were not controlled for. However, with sibship accounted for using maternal identification number as a random effect, all inbreeding effects vanished.

Socioecological variables, such as dominance and pack affiliation, affect telomere length in hyenas (*Proteles cristata*), well known for their strong dominance aggression, with dominant hyenas having significantly longer telomeres (measured using TRF) than psychosocially compromised, lower-ranking individuals.[125] Exposure to predation is likely to similarly cause psychological stress and be comparable to anxiety stress in humans. Indeed, predation vigilance has been suggested to have driven the evolution of anxiety and the perception of fear in humans.[126] In sand lizards, as in many lizard species, an unsuccessful predation attempt may lead to autotomy (tail dropping) and subsequent regeneration. During the regeneration phase, lizards adopt a much more cryptic lifestyle, and bodily growth is stunted in favor of tail regrowth (the regrown tail can be used for further antipredation until the last original vertebra with a fracture plane is lost). However, this comes at a cost in terms of telomere length, but only in males (not in females, as they are camouflaged).[24] Recent work shows that the actual autotomy process itself initiates telomerase upregulation, which would be favored by selection via tail regrowth as a predator-avoidance mechanism.[127]

Interestingly, work on rodent torpor behavior (dormice (*G. glis)*, using qPCR) shows posttorpor telomere attrition in relation to loss in body mass during torpor: the greater the loss in body mass, the greater telomere attrition (using qPCR).[128] Similarly, Turbill *et al.*[129] showed that Djungarian hamsters (*Phodopus sungorus*) lost less or even increased telomere length during torpor. This effect was higher in cold than warm environments and covaried positively with higher food consumption, suggesting a strong link to metabolism. Metabolic or nutritional effects also occur in zebra finches

(*T. guttata*; using qPCR),[130] with attrition of telomere length being associated with sexual maturation in females, but not males, possibly because of different allocation strategies in the two sexes (males allocate micronutrients to the development of sexual coloration). Similar effects can be seen in wild sea birds (*Uria lomvia*), in which the best predictor of among-year variation in telomere length was environmental quality, with longer telomeres in more food-rich colonies and years.[131] Effects of environmental harshness were also found in wild salmon, with shorter telomeres in fish growing in colder water.[132] Research on humans has targeted ill effects of overindulgence and obesity and shown (with marginal significance) that telomere length is negatively linked to obesity in women and high glucose levels in men, but with a positive relationship to high-density lipoprotein (HDL, the "good" cholesterol) in men (using qPCR).[133]

Developmental stress, in the form of greater competition generated by larger clutch size in jackdaws (*C. monedula*), is associated with telomere shortening but not length per se.[134] Similar work by Bateson *et al.*[135] on captive starlings (*Sturnus vulgaris*) confirmed that enlarged clutch size through cross-fostering, in particular when nest mates were heavier, resulted in telomere shortening with concomitant links to behavioral foraging decisions (using qPCR).[135] Whether this is a result of perceived stress (which could be measured as elevation of stress hormones) or simply due to nutritional compromise is unknown. Working on the same assumption—that enlarged clutches increase stress—cross-fostering and brood size manipulations in nestling collared fly catchers (*Ficedulla albicollis*) showed no effect on telomere length, whereas body mass was negatively influenced by increased clutch size.[136] Another experiment on starling nestlings allowed manipulation of size-induced stress (a target chick being larger or smaller than brood competitors), which resulted in telomere attrition independent of growth.[137] However, clutch manipulations with enlarged or reduced clutch size may be hard to interpret when no index of stress is directly measured. Building on these links to developmental stress, work on black-tailed gulls (*Larus crassirostris*) using single nestlings or those with siblings showed that singletons had longer telomeres than birds with sibs.[138] High growth rates were associated with low degrees of telomere shortening, suggesting that high-quality birds were indeed less stressed, grew faster, and better maintained their telomeres.[138] Stress also affects the production of telomerase; for example, in a laboratory rat model (Long-Evans), telomerase levels were 54% higher in stressed than nonstressed rats.[139] Stress was induced (by tilting cages and lightly pinching tails), and the stress outcome was verified with corticosterone assays. Thus, this work convincingly demonstrates that stress, resulting in a physiological stress response, induces telomerase upregulation (verified with Gel-TRAP reactions).

How stress mechanistically causes telomere attrition *in situ* is likely to be a complex, multifactorial process, but one important route is via elevated levels of ROS.[4,106] In agamid lizards, elevated offspring superoxide levels were significantly associated with shorter telomeres (correlative data), even after controlling for maternal telomere length (the strongest predictor of offspring telomere length and significantly heritable).[140] Similarly, in gull chicks (*Larus michahellis*), experimental administration of ingested antioxidants (vitamin E and C cocktail) had a protective effect from oxidation and resulted in longer telomeres in the treatment group (using qPCR).[141] Importantly, the effects of ingested antioxidants on ROS and associated parameters have met with some skepticism in recent years.[142] Endogenous antioxidants (e.g., catalase and superoxide dismutase) may have similar or stronger antioxidative effects, especially since these enzymes evolved in response to oxidation as a selection pressure.[88,143]

Growth rate is a multifactorial trait, and in some cases hormones dictating growth rate may be the real driver of telomere attrition. This is supported by direct manipulation of hormones: in captive zebra finches (*T. guttata*), prelaying females treated with either corticosterone or estradiol exhibited corticosterone-induced stunted growth in sons and telomere loss (measured using qPCR) in mothers and daughters. Sons treated with estradiol gained weight, and oxidative stress was reduced in both sexes, without causing elevated telomere attrition.[144]

In a study of barn swallows (*H. rustica*), a team of researchers screened nestlings for morphometry and sex effects on telomere length during the most intense growth period (days 7–16). Quite extraordinarily, the authors describe a decline in mean

telomere length in little more than a week, with daughters better maintaining telomere length than sons. We are not aware of an estimated life span of red blood cells in barn swallows, but if it is close to that of chickens (28 days) and ducks (42 days),[145] the result could be explained primarily by DNA damage in the red blood cell population at hatching[54] or biased mortality toward red blood cells (and the minority of leukocytes) with long telomeres during just a 9-day period.

Telomerase production in homeotherms has been considered exclusive to germ line and early life stages as an evolved antitumor mechanism. However, work by Haussmann *et al.*[146] clearly demonstrates that long-lived birds with lower rates of telomere shortening have higher telomerase activity in their bone marrow throughout life. In contrast, work on the leatherback turtle (*Dermochelys coriacea*), which exhibits inertial homoethermy due to its large body mass, demonstrates that there is no difference in telomere length (measured using qPCR) between hatchling and old turtles, suggesting a high telomerase activity early in life.[147] A study on tropical lizards[12] (also using qPCR) provides information on a positive, linear relationship between telomerase expression and telomere length; if telomere shortening triggers upregulation of telomerase production, you would expect a negative—not their verified positive—relationship; the unexpected relationship is made even more explicit by flipping the correlation around—the shorter telomeres a lizard has, the less telomerase it produces. However, a lag phase, causing an overshoot of telomerase production subsequent to telomere elongation, could explain such a positive relationship. This result clearly calls for an experimental approach in which telomerase is manipulated and time-lag effects on telomere length and attrition are explicitly teased out. An example of a negative relationship between telomere length and telomerase production is found in rats. Telomere length (measured using TRF) increases in spermatozoa as they develop from the round spermatids in the testes compared with the spermatozoa stored in the epididymis, but telomerase production is lowest in the testes and highest in the epididymis.[148] Thus, the biological pattern during spermatogenesis is opposite to that in Ujvari *et al.*'s[12] study on blood. These differences between studies point to the potential for spurious correlations between telomerase levels and telomere length in different tissues.

Finally, there seems to be no consensus on how various stressors feed into regulatory pathways that govern telomere length. Nevertheless, these mechanisms are part of the mosaic target for phenotypic selection and environmental effects. Stress and physiological perturbations due to single, specific environmental contaminants (such as biomagnifying organohalogenated compounds) have not been proven to correlate with telomere length.[149] However, the summed effect of the urban versus the rural environment has telomere-eroding effects, as demonstrated in a cross-fostering experiment in great tits (using qPCR).[150] Likewise, experimental manipulation of traffic noise showed that telomere length eroded under elevated noise levels without noise having an effect on growth or fledging success (using qPCR).[151]

Quantitative genetics, sex chromosomes, heritability, and evolvability of telomeres

A key aim of this review is to identify evolutionary processes associated with telomere dynamics. In this section, we deliberately deviate from Simons's[89] stringent criteria for acknowledging causality of telomeres in phenomena like aging, longevity, and ultimately lifetime fitness. Whereas Simons looks for immediate mechanistic, molecular effects by the telomere itself on the aging process, we acknowledge all telomeric effects, including regulating effects on other genes or gene products that affect fitness as a correlated mosaic of quantitative traits (thus, epistatic and regulatory effects count, too).

A first indication that selection and drift make populations diverge in telomere characteristics would be to find historical evidence of population genetics processes, such as those between ethnic groups in humans.[25] African American infants have longer telomeres at birth than European American (Caucasian) infants, which researchers have expressed concern for, given that longer telomeres at birth have been associated with more attrition later in life.[152] A number of birth outcomes and demographic factors were controlled for in this study, with the strongest effects observed in daughters (with the longest telomeres by comparison). This said, it seems appropriate to mention that most concern has previously been in regard to having short—not long—telomeres at hatching or birth, and the consequences of this.

In all other fields of evolutionary biology, relative fitness is the "product of survival and reproduction," but, in telomere biology, the second half of this equation, the effect of reproduction as a component of selection (i.e., selection differentials and gradients), has been remarkably overlooked. In this section, we start with the simplistic framework of the breeders equation (response to selection (R) = the narrow sense heritability (h^2) × S (the selection differential)). The reason for this approach is to decompose and make explicit the different processes affecting telomere characteristics during evolution. Importantly, it also prompts us to make explicit the roles (if any) of reproduction, independent of longevity and its aging process, in the evolution of telomere traits. In addition, heritability of telomere traits has been reported for a number of studies in humans as well as other taxa, but often with highly contradictory results. Thus, we have virtually no base on which to formalize thinking and make evolutionary inferences or predictions about telomere dynamics in the wild. Most evolutionary explanations of telomere dynamics invoke selection agents that act after reproductive senescence when selection has no leverage, unless the species in question has grandparental care, as in humans.

In a quantitative genetics framework, short-term evolutionary potential (or evolvability) depends on the additive genetic variance in a population, which is often measured as the narrow-sense heritability ($h^2 = V_A/V_P$)—the fraction of the total phenotypic variance that is additive or, in other words, the parental genetic contribution that, additively, affects the corresponding trait in the offspring.[153] An alternative approach to assessing evolvability is to measure evolutionary potential as the expected proportional change under a unit strength of selection, which yields a mean-scaled additive variance as a measure of evolvability.[154]

Before attempting to understand the heritability and evolvability concepts in the context of evolutionary telomere biology, we need to make a few observations regarding these genetic concepts themselves. To be approximately accurate, the heritability estimate requires random mating, gametic phase equilibrium, absence of additive × additive epistatic genetic variance, and absence of common environmental effects.[155] Furthermore, as used in the breeders equation ($R = h^2 \times S$), the heritability estimate and the selection differential (S) are assumed to be independent in the sense that the response to selection (R) is proportional to their respective values taken in isolation. In fact, R is not, since both h^2 and S depend on the environmental variance, which causes a negative correlation between them. This implies that a large selection differential or high heritability will not necessarily lead to a large evolutionary response.[156]

It was shown recently that the correlation between heritability and evolvability is essentially zero,[156] possibly owing to positive correlations between the additive variance and other components of the phenotypic variance. This means that heritability may be unsuitable as an indicator of evolutionary potential in natural populations, and that wide within- (not to mention among-) species variation in heritability may be influenced by these additional variance parameters (e.g., environmental effects, epistatic effects (gene interactions), and dominance). Therefore, it may not be surprising to see variation in reported telomere heritabilities, which are likely due to differences among populations or taxa in these variance parameters. Given that heritability is a proportional estimate of additive variation in relation to phenotypic variance, it should never be possible to obtain an estimate larger than 1.0 (100%). However, estimation of heritability is, of course, a sampling procedure susceptible to sampling and measurement error like any other. This may contribute to the exceptional estimate by Atema *et al.* (using TRF)[157] of a heritability of telomere length of 99% in a laboratory population of zebra finches and a corresponding estimate in wild sand lizards (*L. agilis*) of more than one (1.23) for son–sire heritabilities and 0.55 for daughter–dam estimates.[71] Given that the telomeres of all sand lizards were sampled and analyzed in the same way, the results clearly suggest a more complex inheritance pattern than simple Mendelian processes.[71] Furthermore, it is important to note that, for sand lizards, the offspring were released at random (geographically) at the study site for over a decade and, hence, there was no environmental confound of the regression effects of mean offspring on parental telomere length,[71] whereas this effect can only be statistically accounted for when parents and offspring share a nesting environment.

In white-throated dippers (*Cinclus cinclus*), Becker *et al.*[158] used an animal model to analyze telomere heritability (using qPCR), Z-linked

inheritance, and environmental effects while controlling for inbreeding effects (Wright's *f*). In spite of strong nest-mate– and mother–offspring resemblance, Becker *et al.*[158] concluded that this resemblance was not explained by additive genetic variance, Z-linkage, or W-linkage (on the basis of strong resemblance between ZW mothers and ZZ sons). It should be noted that, in spite of a relatively large initial sample size ($n = 662$ over 15 generations, 1987–2012), once parentage was traced, the sample sizes dropped quite considerably to an initial 177 individuals for some and 114 individuals for most analyses. Although it was an impressive effort, few studies of natural populations find significant heritabilities for almost any trait with such low sample sizes. In stark contrast to these zero heritability estimates, and with similar sample sizes in a laboratory population (66 females, 57 males, and 73 broods), Atema *et al.*'s[157] 0.99 heritability estimate using TRF was indistinguishable from one (1.0). In another bird species, (king penguins, *Aptenodytes patagonicus*), maternal heritability of telomere length (measures using qPCR) was significant between mothers and chicks early in life, but was no longer significant as offspring became older.[159] Another example of maternally linked heritability in a bird species is that of the kakapo (*Strigops habroptilus*) from New Zealand,[160] which also showed longer telomeres (using TRF) in males than females but with no relationship to age (exact heritability estimate not given).

In humans, the most commonly studied species, heritability estimates predominantly come from twin studies[161,162] (but see Ref. 163). In medicine, twin studies have been seen as the gold standard for differentiating between a trait's genetic and environmental components since the late 19th century, when the Scottish obstetrician J. Matthews Duncan suggested that there were two types of twins, later suggested by Francis Galton as a useful scenario for teasing out genetic versus environmental trait variation.[164] In classic twin studies, monozygotic (MZ) and dizygotic (DZ) intraclass correlations for a trait are compared, and a significantly higher correlation in MZ twins suggests a genetic effect on the target trait. Thus, genetic determinism of telomere length is supported by the finding that monozygotic twins have more similar telomere attributes than dizygotic twins.[117] In order to estimate the heritability of the trait (i.e., the proportion of the population variance due to additive genetic variation), and ignoring higher-order epistatic gene actions that are often considered beyond statistical reach,[155] Bischoff *et al.*[40,162] assumed that the total variance in telomere length in the population could be decomposed as $V = A + D + C + E$, where V equals the total population variance in telomere length, A refers to the variance contribution of additive genetic effects, D refers to the variance due to dominance (intralocus interactions), C refers to the variance contribution of shared environmental effects (i.e., environmental factors that are shared by reared-together twins and are thus the source of their similarity), and E refers to the variance due to nonshared environmental effects (i.e., environmental effects that are not shared by reared-together twins and are thus a source of dissimilarity). If so, and assuming that shared environmental effects contribute equally to the resemblance of MZ and DZ twins, the expected twin covariances are given by $\mathrm{cov(MZ)} = A + D + C$ and $\mathrm{cov(DZ)} = (1/2)A + (1/4)D + C$.

For analytical details, see Bischoff *et al.*,[162] but, in brief, variance components were estimated from the observed twin variances and covariances, and heritability was calculated to be 0.36, with no difference between males and females. Thus, if estimates of inheritance and selection coefficients relating to telomere traits show bias, we need to understand why and how they may confound or complicate evolutionary inference. One reason for differences in heritability among taxa may be the use of different techniques for estimating breeding equation components, such as heritabilities, using twin studies, animal models, or mid-offspring–mid-parent regressions. For instance, the difference in estimated variance between monozygotic twins in shared versus nonshared environments will always be near impossible to obtain data for in other taxa in natural populations. Furthermore, recent work by Asghar *et al.*[112] shows that telomere length in offspring increased with maternal age in hatchlings from malaria-uninfected female reed warblers, whereas offspring from malaria-infected mothers had shorter telomeres as mothers got older (Fig. 6). Elsewhere, Asghar *et al.*[165] showed maternal (but not paternal) heritability ($h^2 = 1.08$ for mothers, 0.28 for fathers) for telomere length. Thus, this may suggest that age-dependent telomere loss due to disease may affect not only the

heritability estimate but also evolutionary inference on the basis of the breeder's equation (see also Ref. 175 for a recent overview of h^2 estimation in the wild).

Quantitative trait loci analysis has shown that variation in the DNA helicase gene *DDX11* on chromosome 12 in humans explains 49% of overall variability in telomere length and that this contributes strongly to the 81.9% telomere heritability found by Vasa-Nicotera *et al.*[166] Other work shows that X-linked inheritance is the most likely explanation of sex-biased heritability in humans between fathers and daughters, mothers and sons, fathers and sons, and between siblings—but with no correlation between telomere lengths of spouses.[167] Nongenetic (epigenetic) factors also seem to contribute to telomere length, as indicated by accelerated telomere shortening in the inactive X chromosome (X_i).[168] A possible mechanistic explanation to this would be that, since the X_i telomeres are part of the Barr body and less available to telomere-maintenance mechanisms, such as telomerase and recombination, comparing X_i and X_a (activated X) gives an indication of the efficiency of these processes for maintaining telomere length.

Transgenetic and epigenetic effects on telomere traits

One tissue type of fundamental importance in evolutionary biology is spermatozoa, the source of paternal genetic and potentially epigenetic inheritance. Interestingly, telomeres are modified by ROS, influence offspring fitness through vertical transmission of genetic material, and have direct, proximate effects on the swimming ability of sperm.[18] Standard swim-up trials used to select the best sperm for assisted reproduction in humans show that faster-swimming sperm have longer telomeres and less DNA breakage.[18] Interestingly, telomeres are longer in sperm from older men, which thus follows the same age trend as telomeres in blood (using TRF).[42] In sand lizards, however, Olsson *et al.* (using TRF)[71] showed that paternal age is negatively correlated with offspring telomere length, suggesting that sperm telomeres shorten through life. Work on mice showed that telomeres are longer in spermatozoa than in oocytes, that old males have shorter telomeres than younger males, and that offspring from younger males have longer telomeres (using qPCR).[169] Other work on mice also demonstrates that telomere elongation in the haploid sperm cells occurs after meiosis and in the absence of genomic replication and, hence, represents an alternative telomere-extension pathway (using Q-FISH and TRAP).[170] Furthermore, telomerase gene null mice exhibit progressive defects in highly proliferative tissues and decreased fertility and, ultimately, sterility.[171] These mice studies also indicate that germ cells lacking telomerase ultimately lose telomere base pairs or entire telomeres, resulting in aberrant fertilization, partly due to sperm DNA fragmentation[172] and abnormal cleavage of embryos. Thus, this indicates an important role for functional telomeres in germ cells undergoing fertilization and cleavage development.[171]

Conclusions

In summary, we review the literature on telomere biology from the perspective of evolutionary ecology and genetics. We find strong correlative links between telomere length, telomere attrition, and selection pressures, such as those associated with phenotype-compromising effects on reproduction from cancer, atherosclerosis, and other disease—all at least potentially debuting before reproductive senescence. Evidence for directly causal effects of telomere traits on life history and fitness parameters is limited. Heritability estimates of telomere traits vary dramatically across studies and taxa, and perhaps some of this variation is due to differences in estimation techniques, such as mid-offspring–mid-parent regressions, animal models, and (mostly in humans) twin studies. To what extent this picture is biased from unpublished null results is not known. There are considerable publication records on robust research using both qPCR and TRF (Southern blotting) in evolutionary ecology and genetics. Future work in evolutionary ecology on telomeres needs to embrace work on nonmodel taxa in natural populations, incorporating the effects of telomere traits on reproductive parameters. In particular, species with telomerase production in somatic tissue may be suitable models for understanding selection pressures from disease susceptibility (e.g., cancer) associated with variation in telomerase production and associated variation in cancer risk. We encourage researchers to pursue studies of interstitial telomeres, since these have been linked to elevated recombination rate and a number of fitness-compromising effects.

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Competing interests

The authors declare no competing interests.

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