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INVITED REVIEW

Measuring vertebrate telomeres: applications and limitations

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Abstract

Telomeres are short tandem repeated sequences of DNA found at the ends of eukaryotic chromosomes that function in stabilizing chromosomal end integrity. *In vivo* studies of somatic tissue of mammals and birds have shown a correlation between telomere length and organismal age within species, and correlations between telomere shortening rate and lifespan among species. This result presents the tantalizing possibility that telomere length could be used to provide much needed information on age, ageing and survival in natural populations where longitudinal studies are lacking. Here we review methods available for measuring telomere length and discuss the potential uses and limitations of telomeres as age and ageing estimators in the fields of vertebrate ecology, evolution and conservation.

Keywords: age, ageing, biological age, chronological age, estimator, telomere length, telomere shortening

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Introduction

We all know that age affects the way organisms function and is critically important in determining an individual's physiological state and response to environmental conditions (Ricklefs 1977; Stearns & Koella 1986; Stearns 1989; Lindstrom 1999; Ricklefs & Wikelski 2002). Age affects risk of injury and disease, probability of survival and reproductive potential, and is a major determinant of population growth potential (Stearns & Koella 1986; Albon *et al*. 2000; Coulson *et al*. 2001; Kern *et al*. 2001). Despite its importance, age structure is seldom incorporated into population models, yet we know that knowledge of age provides important insights into population and evolutionary processes that would otherwise remain hidden (e.g. how reproductive output, offspring sex and offspring viability vary with age) (Stearns & Koella 1986; Stearns 1989; Charnov 1991; Lindstrom 1999; Kern *et al*. 2001).

In most natural populations the age of individuals is rarely known because these data are impractical or, as yet, impossible to obtain. Extensive longitudinal study of marked individuals provides one means of obtaining such data, but generally these data come at significant cost and cover only a fraction of the study population. Morphological correlates of age may also be useful, but these approaches (e.g. counting otolith growth rings in fish, measuring wax plugs from the inner ears of whales) often require destructive sampling. Recently it has been suggested that telomere length may provide a powerful new tool for estimating age in natural populations where longitudinal data are limited (Haussmann & Vleck 2002; Vleck *et al*. 2003).

Telomeres are short tandem repeated sequences of DNA found at the ends of eukaryotic chromosomes that function in stabilizing chromosomal end integrity (Box 1, Fig. 1). Haussmann and colleagues (Haussmann & Vleck 2002; Haussmann *et al*. 2003a; Vleck *et al*. 2003) have demonstrated recently that it might be possible to estimate the age of animals by measuring telomere lengths because telomeres in somatic tissues normally shorten with age. They have shown that telomere (or terminal) restriction fragments (TRF) from blood samples in some avian species shortened with age [e.g. zebra finches, *Taeniopygia guttata* (Haussmann & Vleck 2002) and common terns, *Sterna hirundo* (Haussmann *et al*. 2003a)]. They also found that the rate of telomere shortening (telomere length rate of change; TROC) was

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Box 1. Telomeres, factors regulating telomere length, and aging

Telomeres comprise many copies of an evolutionarily conserved DNA repeat, found at the natural ends of all linear eukaryotic chromosomes. The repeats consist of a short G-rich sequence; in vertebrates, the telomeric repeat $(TTAGGG)_n$ is conserved (Meyne *et al*. 1989) (Fig. 1). This natural end, along with associated proteins, provides chromosome stability, preventing degradation and chromosome fusion (while also anchoring chromosomes in the nuclear matrix); chromosome ends created by breakage are prone to fuse with other chromosomes (Blackburn 1991; Greider 1996). Telomere-associated proteins such as TRF1 and TRF2 are also involved in the regulation of telomere length (Fig. 1) (Broccoli *et al*. 1997; van Steensel & de Lange 1997; Karlseder *et al*. 2002). Telomeres play an essential part in DNA replication. At each cell division, a small number of telomeric repeats is lost because DNA replication is incomplete at the 3′ end of the double strands (i.e. the end-replication problem) (Watson 1972), leaving G-rich strand overhangs [whose length is a determinant in the rate of telomere shortening (Huffman *et al*. 2000)].

Telomerase is a ribonucleoprotein reverse transcriptase that restores telomere repeats (Lingner *et al*. 1997). Telomerase activity is found in continually proliferating germ cells and stem cells, but for other types of somatic cell, various or zero activity levels are observed in different species (Prowse & Greider 1995; Venkatesan & Price 1998). The shortening of telomeres has been suggested to be one of the main mechanisms underlying ageing and age-related diseases, because loss of telomere function can lead to genome instability and cell replicative senescence (Harley *et al*. 1992; Campisi 1996). Mechanistic explanations of ageing involve the accumulation of mutations in genes, the shortening of telomeres and damage in mitochondrial DNA, in all of which oxidative damage plays a crucial role (Goyns 2002) [for free radical theories of ageing, see Finkel & Holbrook (2000)]. Oxidative stress incurred by reactive oxygen species (ROS) increases the rate of telomere shortening per cell division and also the rate of cell turnover (Fig. 1).

Interestingly, in humans, telomere shortening (Benetos *et al*. 2001) occurs at different rates in males and females, presumably as a response to different levels of oestrogen between the sexes, which can stimulate telomerase activity and attenuate ROS (Aviv 2002b). Sex differences in telomere shortening have not been observed in any other species, but the possibility needs to be considered if telomere length is to become a tool useful for molecular ecological studies.

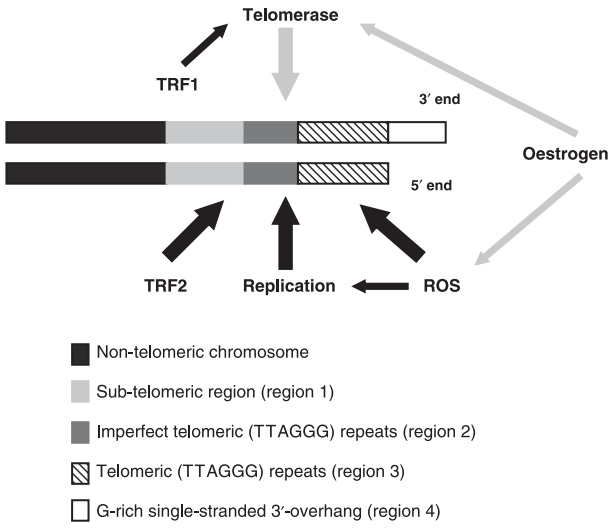

Fig. 1 Vertebrate telomere structure (region codes are used in Table 1) and factors regulating telomere length: direct factors (large arrows), indirect factors (small arrows), factors accelerating telomere shortening (black arrows) and factors attenuating telomere shortening (grey arrows). TRF1 and TRF2 are telomere-associated proteins and ROS (reactive oxygen species) are the main source of oxidative stress. Adapted and modified from Aviv (2002b) and Sedivy *et al*. (2003).

correlated with lifespan (also known as a maximum lifespan) in birds and mammals (Haussmann *et al*. 2003b; Vleck *et al*. 2003) (Box 2). They suggested that variation in telomere length rate of change (TROC) might be a molecular mechanism underlying the evolution of species lifespan (or ageing), which appear to be species-specific and genetically determined (Campisi 1996; Turker & Martin 1998).

Inevitably, for many wild populations information on age is lacking, as the time and effort usually required to determine individual ages and age structure in study populations is often prohibitive. Nevertheless, the insight that such knowledge provides into the ontogeny of life history traits and population dynamics is recognized widely (Coulson *et al*. 2001; Vleck *et al*. 2003). Telomere length assays may provide a direct and cost-effective approach to obtaining immediate knowledge of age, age structure and ageing in wild populations and represent a major leap forward in the molecular arsenal at the disposal of ecologists. This technology may prove to be as revolutionary as earlier DNA-based approaches to parentage analysis (Jones & Ardren 2003) and molecular sexing (Ellegren & Sheldon 1997). We anticipate that just like these earlier approaches, telomere

Box 2. Telomere length, telomere length rate of change and lifespan in birds and mammals

Maximum telomere length and the telomere length rate of change (TROC) differ among species, as does maximum lifespan among species (Table 2 and Fig. 2). Haussmann and colleagues (Haussmann *et al*. 2003b; Vleck *et al*. 2003) found that telomere length at a given life stage did not correlate with lifespan but TROC correlated with lifespan in birds and mammals (Fig. 2c). Telomere length and TROC vary among individuals of the same species and among tissues from an individual (Table 1). Inter-species, interindividual and intertissue differences are accounted for mainly by factors such as different rates of cell replication, levels of telomerase activity and levels of oxidative stress (Fig. 1) (Aviv 2002b). It is particularly interesting that the telomeres of Leach's storm-petrels (*Oceanodroma leucorhoa*), an unusually long-lived species, lengthen with age (Fig. 2b), because lengthening of telomeres (i.e. telomerase activity) is associated usually with cancer [cancer cells are immortalized through telomerase activity or alternative processes that lengthen telomeres (Henson *et al*. 2002; Blasco 2003)]. It is proposed that the low or zero telomerase activity found in somatic cells may have been selected for to reduce the frequency of cancer (Harley *et al*. 1994). How Leach's storm-petrels avoid the tumour-susceptibility imposed by telomerase activity is of considerable potential interest in medical research (Haussmann *et al*. 2003b).

Despite the apparently adverse conditions promoting ageing in birds, such as high oxygen consumption and high body temperature, avian species tend to live longer than mammals of comparable body sizes. For a given body size, birds produce less reactive oxygen species (ROS), which are one of the main sources of oxidative damage, and are more tolerant to oxidative damage than mammals (Ogburn *et al*. 2001). Also, in several mammalian species the production of ROS and levels of oxidative damage are higher in short-lived spices than long-lives ones (Barja & Herrero 2000). Clarification of the relationships between telomerase activity and both oxidative damage and lifespan in a range of avian species is currently under investigation (Vleck *et al*. 2003).

Table 1 Selected methods available for measuring telomeric regions

Method[a]	Hybridization[b] or PCR-based method?	No. of cells required[c]	Telomeric regions measured[d]	Appropriate for blood samples?	Average of single or all sample cells?	Average of single or all chromosomes?	Refs
TRF analysis	Hybridization	Large	2, 3, 4 (1)[e]	Yes	All	All	Harley *et al*. (1990)
Q-PCR	PCR-based	Small	2, 3, 4	Yes	All	All	Cawthon (2002)
Q-FISH	Hybridization	Intermediate	2, 3, 4	No	Single	Both	Zijlmans *et al*. (1997)
Flow-FISH	Hybridization	Intermediate	2, 3, 4	Yes	Single	All	Rufer *et al*. (1998)
STELA	PCR-based	Small	2, 3 (1)[e]	Yes	All	Single	Baird *et al*. (2003)
T-OLA	Hybridization	Large	4	Yes	All	All	Cimino-Reale *et al*. (2001)

[a]Abbreviations: terminal (or telomere) restriction fragment (TRF), quantitative fluorescence *in situ* hybridization (Q-FISH), flow cytometry method using fluorescence *in situ* hybridization (Flow-FISH), telomeric–oligonucleotide ligation assays (T-OLA), real time kinetic quantitative ploymerase chain reaction (Q-PCR), single telomere length analysis (STELA).
[b]Methods using probes hybridize TTAGGG-repeat sequence.
[c]Cell quantities for assays are taken from Sedivy *et al*. (2003). As a guide, TRF requires 5–10 μg of DNA, while STELA requires 50–100 ng of DNA.
[d]Region codes are from Fig. 1: 1 = subtelomeric, 2 = imperfect telomeric repeats, 3 = telomeric repeats, 4 = G-rich single-stranded 3′-overhang.
[e]Part of the region 1 (subtelomeric region) is measured.

length assays will open up new avenues and research opportunities in the fields of ecology, evolution and conservation.

In this article, we review the methods available for measuring telomere length and TROC and explore the use and limitations of vertebrate telomere assays in the field of ecology, evolution and conservation.

Measuring telomere length and TROC

Telomere restriction fragment assays

There are a number of techniques available for measuring telomere length (Table 1). The telomere (terminal) restriction

Table 2 Lifespan, telomere length, and telomere length rate of change (TROC) in birds and mammals from selected published literature[a]

Species	Maximum lifespan (years)[b]	Maximum observed telomere length (bp)[c]	TROC (bp/year)	Tissue sampled	Refs
Zebra finch (*Taeniopygia guttata*)	5	9300	–515	Erythrocyte	Haussmann *et al*. (2003b)
Tree swallow (*Tachycineta bicolor*)	11	17 300	–391	Erythrocyte	Haussmann *et al*. (2003b)
Adélie penguin (*Pygoscelis adeliae*)	20	9500	–235	Erythrocyte	Haussmann *et al*. (2003b)
Common tern (*Sterna hirundo*)	26	9800	–57	Erythrocyte	Haussmann *et al*. (2003b)
Leach's storm-petrel (*Ocenodroma leucorhoa*)	36	20 000	75[d]	Erythrocyte	Haussmann *et al*. (2003b)
Western wild mice (*Mus spretus*)	3.5	9500	–600	Spleen	Coviello-McLaughlin & Prowse (1997)
Cattle (*Bos taurus*)	30	22 000	–230	Leucocytes	Miyashita *et al*. (2002)
Cynomolgus monkey (*Macaca fascicularis*)	37	16 500	–63	Leucocytes	Lee *et al*. (2002)
Human (*Homo sapiens*)	110	10 000	–33	Leucocytes	Hastie *et al*. (1990)
Human (*Homo sapiens*)	110	10 500	–15	Fibroblasts	Allsopp *et al*. (1992)
Human (*Homo sapiens*)	110	9000	–68	Stem cells	Vaziri *et al*. (1994)
Human (*Homo sapiens*)	110	20 000	71[d]	Sperm	Allsopp *et al*. (1992)

[a]Modified from Haussmann *et al*. (2003b).
[b]From Haussmann *et al*. (2003b) and references therein.
[c]These are approximate values observed in the study and the values come from different age stages (mostly at the age of zero).
[d]Telomere length increased with age in these cells.

fragment (TRF) analysis (Harley *et al*. 1990) and related methods such as the telomere amount and length assay (TALA) (Gan *et al*. 2001) are relatively easy methods and are probably the most widely used in telomere research. In these methods, average lengths of TRFs (created by particular restriction enzymes and hybridized with a radioactive oligonucleotide) are measured (see Harley *et al*. 1990). One major drawback of this approach is that the lengths of TRFs usually differ between cells and chromosomes because cells may differ in age and different chromosomes have different restriction sites relative to telomeric ends. Consequently, TRF assays produce autoradiographic smears which have some element of subjectivity in their analyses, although there is software available to remove some arbitrary decision making (Grant *et al*. 2001). Furthermore, the TRF method requires relatively large amounts of DNA (10 μg) and time, and TRF length differences between individuals can differ as much as 5%, depending on which restriction enzyme is used (indicating that subtelomeric restriction site polymorphisms and /or subtelomeric length polymorphisms may exist) (Cawthon 2002).

Quantitative polymerase chain reaction (PCR) assays

Real-time kinetic quantitative PCR (Q-PCR) measurement of telomere length (Cawthon 2002) avoids many of the problems encountered by TRF analysis by using ingeniously designed primers that hybridize to vertebrate telomeric regions without generating primer dimer-derived products. This method measures relative telomere lengths by determining the factor by which a sample DNA differs from an arbitrary reference DNA in its ratio of telomere repeat copy number (T) to single gene copy number (S) (i.e. relative T/S ratio). The T/S ratio of one individual relative to the T/S ratio of another should reflect relative telomere length differences between individuals. This method is very useful for determining interindividual differences in telomere length within a species and may also be used to measure relative telomere length among species if those species share the same single copy reference sequence. In theory, any single copy gene sequence can be used for standardization, so the technique should be applicable to species even where genetic data are limiting.

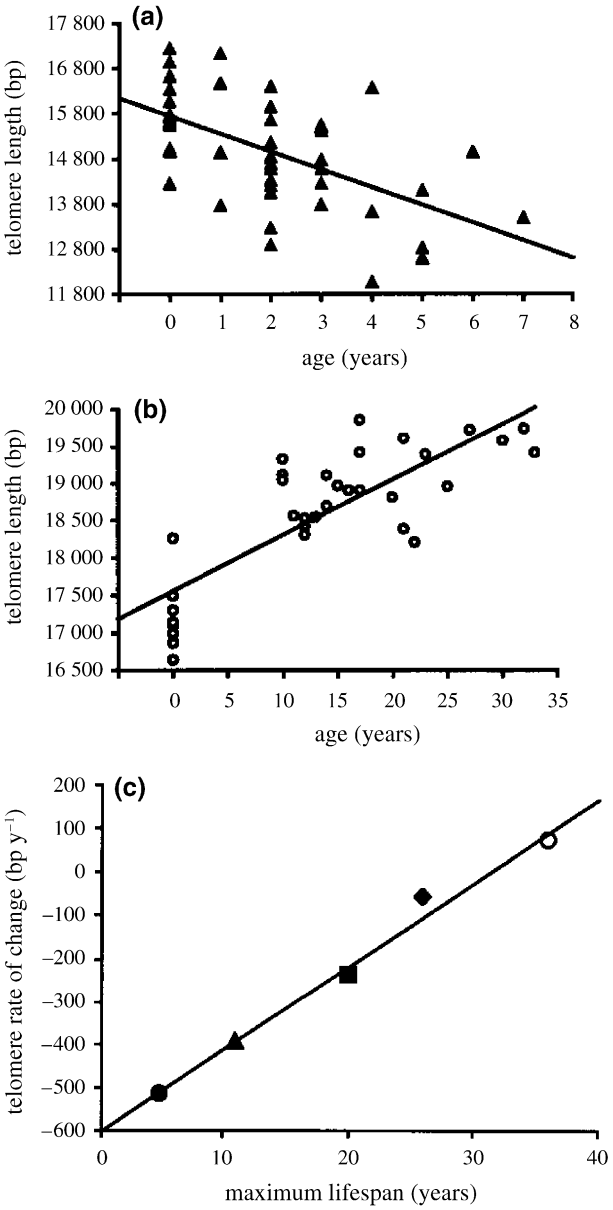

Fig. 2 Telomere length (measured by telomere restriction fragment length) as a function of age (a) in tree swallows (slope = −391 ± 65 (SE) bpy^{-1}, $r^2 = 0.34$) and (b) in Leach's storm-petrels (slope = 75 ± 10 (SE) bpy^{-1}, $r^2 = 0.66$). (c) The telomere length rate of change as a function of maximum lifespan in birds (slope = 19.5 ± 1.2 (SE) bpy^{-1}, $r^2 = 0.99$): zebra finches (black circle), tree swallows (triangle), Adélie penguins (square), common terns (diamond) and Leach's storm-petrels (open circle). Adapted from Haussmann *et al*. (2003b).

Despite its strengths, Q-PCR has two problems: (1) it does not provide absolute telomere lengths and (2) if target species have interstitial telomeric sequences [birds have telomeric repeats in the centromeric region (Venkatesan & Price 1998)], Q-PCR will measure interstitial telomeric repeats as well as terminal telomeric repeats. The former problem is resolved by using both TRF analysis (Harley *et al*. 1990) and Q-PCR for a portion of the samples so that the change in length in base pairs (bp) of DNA per unit change in the T/S ratio can be determined, but the latter will be problematic if there is large variation in interstitial telomeric sequences among individuals or species. However, this is not a critical issue if one is interested in measuring only telomere length rate of change, because the extent of interstitial telomeric repeats should not change. Furthermore, it may be possible to estimate the extent of interstitial telomeric sequence by using Q-PCR in combination with TRF analysis and Bal31 exonuclease digestion, which will preferentially digest terminal telomeric sequences.

Fluorescence in situ *hybridization (FISH) methods*

Quantitative fluorescence *in situ* hybridization (Q-FISH) (Zijlmans *et al*. 1997) and flow cytometry methods using fluorescence *in situ* hybridization (Flow-FISH) (Rufer *et al*. 1998) are also often used to measure telomere length. As their name suggests, fluorescent dyes are used to visualize telomeres which are quantified by measuring light intensity. The FISH methods are able to measure telomere lengths of single cells. Flow-FISH uses fluorescence-activated cell sorting (FACS) to sort different types of cells, while Q-FISH provides measurements of telomere lengths of individual chromosomes. There is some evidence that the shortest telomere, rather than the average, is critical for loss of telomere function (Hemann *et al*. 2001), although average length might correlate with shortest telomere length. However, because Q-FISH visualizes telomeres in metaphase chromosomes tissue types used need to be proliferating cells, which limits its suitability for studies of wild populations, as blood or tissues would need to be collected and processed with a view to generating adequate numbers of metaphase chromosomes. Furthermore, the FISH methods, especially Q-FISH (Sedivy *et al*. 2003), are difficult to establish in nonspecialist laboratories so we think that there will be few cases where the high-resolution approaches of the FISH methods would be applied in ecological, evolutional and conservational work.

Single telomere length analysis (STELA)

STELA (single telomere length analysis), a recently devised PCR-based method that measures the lengths of individual telomeres (Baird *et al*. 2003), also merits consideration. Baird *et al*. (2003) reported marked telomere length differences between alleles (some are much shorter than others) in human sex chromosomes and speculated that these differences might account for significant differences in the rate of ageing among human populations (Perls *et al*. 2002). Currently, this method is not readily applicable to most

species because the DNA sequences of subtelomeric regions need to be known. However, with sequence information coming on line from a host of genome projects, this approach will soon be applicable to primates, rodents, ungulates, carnivores, galliform birds and frog species related closely to *Xenopus*. We think that a generalized STELA assay for vertebrate telomeres might not be far away.

Telomeric–oligonucleotide ligation assays (T-OLA)

T-OLA measure the length of the G-rich telomeric-3′-overhang by ligating telomeric repeat oligonucleotides hybridized to the overhang (Cimino-Reale *et al*. 2001). Telomere shortening is proportional to the size of G-rich overhangs in human tissues (Huffman *et al*. 2000), and this observation might extend to other vertebrates. Although it has yet to be tested explicitly, given the correlation between TROC and lifespan (Haussmann *et al*. 2003b), it might be expected that long-lived species (i.e. species with slow telomere shortening) will have short G-rich overhangs while short-lived species will have long G-rich overhangs (Huffman *et al*. 2000). However, the abundance of short overhangs may also be proportional to the number of cells that have entered cellular senescence (Stewart *et al*. 2003). Consequently, it is currently unclear whether short overhangs mean slow telomere shortening (perhaps predicting longer lifespans) or many senescent cells (perhaps predicting shorter remaining life).

Sampling for telomere assays

In order for telomere assays to become a common tool in vertebrate ecology we will need to be able to sample somatic cells that are subject to constant division, and to possess a widely applicable method which can be used to measure telomere length efficiently in many individuals. Haussmann & Vleck (2002) suggest blood as an excellent candidate tissue for two reasons: (1) blood samples can be obtained easily from a wide variety of species and are often collected for other reasons and (2) telomeres in blood cells may shorten at a greater rate than those of other tissues (Hastie *et al*. 1990) because of their high turnover rate (Chang & Harley 1995). In nonmammalian vertebrates, blood samples of 50 μl may contain adequate numbers of nucleated red blood cells, from which telomere length can be obtained. For mammals, whose erythrocytes lack nuclei, telomere length can be measured in white blood cells, albeit with a need to draw larger blood volumes to account for the smaller numbers of white vs. red blood cells. However, there is no reason to restrict ourselves only to the use of blood samples; tissue biopsy samples taken routinely for many large vertebrates (e.g. Lambertsen 1987; Gemmell & Majluf 1997) might well prove to be adequate for telomere analyses.

Telomere shortening among and within species

Haussmann and colleagues (Haussmann *et al*. 2003b; Vleck *et al*. 2003) demonstrated that the rate of shortening varies among species, and that lifespan correlates with TROC in birds and mammals (Box 2). They suggest that lifespan and regulation of telomere length may have coevolved so that the replicative lifespan of individual somatic cell lineages increases with lifespan (Vleck *et al*. 2003). There are two major evolutionary theories of ageing the antagonistic pleiotropy model and the mutation accumulation model (Rose 1991; Kirkwood & Austad 2000; Partridge & Gems 2002). The former model predicts that ageing has evolved as a result of pleiotropic alleles which are beneficial for survival and reproduction early in life, but that are detrimental for survival and reproduction later in life, being able to accumulate in populations. The latter model predicts that ageing has evolved as a result of alleles with detrimental effects being able to accumulate in populations if they are expressed only later in life when selection pressure is weak. Both theories predict that if the extrinsic rate of mortality increases, selection against senescence will be weak and biological ageing will accelerate (so decreasing lifespan). It is possible that TROC may be, in part, a molecular mechanism that contributes to the rate of ageing and thus of lifespan, as suggested by Haussmann *et al*. (2003b). Examination of TROC in more species will help to clarify this hypothesis.

Differences in TROC among individuals of the same species have so far had very little attention. If telomere length were seen as an age estimator, TROC could be used to estimate the rate of ageing, which may be compared with other life history traits. Potentially, we could ask interesting questions regarding life history trade-offs — for example, is the rate of telomere shortening higher in hard-working parents than lazy ones (i.e. is there some trade-off between energy expenditure and ageing)? TROC may well be different among populations of the same species living in different environmental circumstances, which may in turn influence the level of reactive oxygen species (ROS) (Fig. 1). Such a scenario would enable the investigation of the effects of pollutants or climate on ageing — for example, is TROC different between populations in and around Chernobyl when compared to other sites?

Recent studies, however, suggest that the rate of telomere shortening changes over time (Frenck *et al*. 1998; Rufer *et al*. 1999; Zeichner *et al*. 1999), which may impose some limitations on studies comparing TROC among individuals. For example, it has been reported in humans that telomere shortening in leucocytes occurs rapidly in young children, followed by an apparently stable period (little shortening) between age 4 years to young adulthood, and by gradual shortening in later life (Frenck *et al*. 1998; but see Rufer *et al*. 1999; Zeichner *et al*. 1999). It is still not clear

whether differences in cell turnover rate or in the regulation of telomere length over different stages of life (or both) contribute to this change over time (Frenck *et al*. 1998; Rufer *et al*. 1999; Zeichner *et al*. 1999; Friedrich *et al*. 2001; Brummendorf *et al*. 2002; Sidorov *et al*. 2003). It is likely that the change in telomere shortening over time differs considerably among species.

Estimating chronological or biological age?

Telomere length as an age estimator is promising, but it is not without problems. There is great variation in telomere length among individuals of the same age in a number of bird species (Haussmann & Vleck 2002; Haussmann *et al*. 2003a,b). Similar variation has been described in some mammalian species [e.g. humans, *Homo sapiens* (Hastie *et al*. 1990; Allsopp *et al*. 1992) and western wild mice, *Mus spretus* (Coviello-McLaughlin & Prowse 1997)]. The variation in telomere length results from a combination of variation in initial telomere length and thereafter in TROC. Telomere length therefore correlates with chronological age but does not always predict it reliably (e.g. a 1-year-old tree swallow had a shorter telomere length than one 6 years old; Fig. 2). Also, the reported variations in TROC suggest further limitations. For example, telomeres lengthen with age, albeit linearly, in Leach's storm-petrels, *Oceanodroma leucorhoa* (Box 2). In some species telomere length may change very little or not at all over an entire lifetime, as observed in the European freshwater turtle *Emys orbicularis* (living more than 100 years in captivity), which showed no significant change in the telomere length between embryos and adults (Girondot & Garcia 1998).

Species suitable for estimating chronological ages are probably those with a large change in telomere length over their lifetime and small variation in initial telomere length. For example, in humans accurate age estimation could be achieved from blood and dental pulp samples for forensic proposes (Tsuji *et al*. 2002; Takasaki *et al*. 2003). If such approaches can satisfy the rigorous requirements of forensic science, then there may be other cases in which chronological age estimation by telomere length may find a role. However, for ecological and evolutionary purposes, telomere length might be better employed as an indicator of 'biological age' than as an estimator of chronological age (Aviv 2002a; Harley *et al*. 1992).

Biological age is expressed by variation in lifespan among species and individuals of the same spices. Telomere length reflects genome stability (Blackburn 1991; Greider 1996) and is highly heritable in humans (Slagboom *et al*. 1994; Jeanclos *et al*. 2000). As telomeres shorten at each cell division, telomeres are supposed effectively to record the replicative history of somatic cells in vertebrates (Aviv 2002a,b; Goyns 2002). Moreover, recent work has show that oxidative damage (i.e. reactive oxygen species, ROS) may be the main cause of telomere shortening (von Zglinicki *et al*. 2000) (Fig. 1). Oxidative stress is incurred both from the environment and from endogenous metabolic processes (Goyns 2002), so that telomere length reflects deleterious environmental factors to which an organism has been exposed, as well as genetic factors. Variation in longevity in the members of the same species may therefore be explained in part by biological age, as revealed through telomere length. Support for the proposition that this concept of biological age may be more important than chronological age comes again from humans, in which Cawthon *et al*. (2003) demonstrated that individuals who had shorter than average telomeres at the age of 60 or older suffered more from diseases of old age later in their lives.

Telomere assays of biological age as a tool in ecology

The direct use of biological age as a trait in ecological, evolutionary and conservational studies may prove particularly fruitful. For example, in Soay sheep older sheep respond differently to weather and density affects than younger sheep, yet previous population models had not dealt specifically with the issue of age structure (Coulson *et al*. 2001). When chronological age structure was incorporated into population models for Soay sheep the ability of these models to predict population crashes was improved dramatically (Coulson *et al*. 2001). How would this model perform if chronological age was substituted with the biological age of sheep? Our prediction is that chronological age may reflect more an individual's experience and learned behaviour, while biological age may be more representative of some aspect of individual physical condition. While both age concepts will overlap considerably we suspect that, for many of the life history issues molecular ecologists wish to address, biological age may be more meaningful.

The use of telomere length as an indicator of biological age will probably be limited to species whose telomere length changes over their lifetime (for most vertebrate species studied, telomere length shortens over a lifetime). It should also be noted that deleterious physical condition caused by short telomeres (e.g. replicative senescence and age-related diseases) may occur only in particularly long-lived species such as humans (Aviv 2002b). In most wild animals it seems unlikely that telomere function (Box 1) will be lost during an animal's lifetime, despite senescence being a common phenomenon among species; (Kirkwood & Austad 2000; Partridge & Gems 2002), because most animals have a much higher extrinsic mortality rate than humans. None the less, telomere assays may, finally, provide opportunities to enable ecologists to gain data on age specific survival rates and age-specific reproductive rates in species where such data are hard or previously impossible to obtain. We believe that there is great potential for the future application of this technique to open up new possibilities

Box 3. Telomere length as a tool in vertebrate conservation management

Telomere length assays may revolutionize the management of many endangered vertebrate species, where data on age may be lacking, but such knowledge might enhance dramatically predictive models and conservation recovery plans. This is especially true of long-lived k-selected vertebrate species that are grossly over-represented in the IUCN *Red Book* (IUCN 2003). The critically endangered kakapo (*Strigops habroptilus*) is an archetypical representative of this type of species. Kakapo are currently returning from near extinction after the discovery of remnant populations in Fiordland and Stewart Island in the 1970s (Elliott *et al*. 2001). In 2003 the population consisted of 86 individuals. However, none of the reproductively active individuals in the current population are of known ages (Elliott *et al*. 2001). With suggestions that some of the breeding birds may be 60+ and possibly older, and with demonstrably different levels of breeding success among this population (Miller *et al*. 2003), it would be useful to know what the age structure of this population is and how reproductive performance varies across individuals' life spans in order to better predict the future trends of this species. Furthermore, knowledge of age, when used in conjunction with traditional molecular markers, such as microsatellites, would also enable the elucidation of family groups and pedigrees where these are unknown, greatly improving our understanding of the social organization and dynamics of species that have not been subject to long-term study. Given that blood samples of adequate size (> 50 μl) are available for every kakapo, it is possible to use both TRF and Q-PCR assays to provide the knowledge of age needed to help better manage kakapo recovery.

The application of this technology to other species is no less impressive. For example, new molecular tools for ageing together with other established approaches might see an end to lethal sampling programmes such as the scientific whaling programme (Brownell *et al*. 2000; Aron 2001). In the past a desire to understand the age structure of natural populations might appear to be one of the few legitimate areas where lethal sampling might still be justifiable. However, if validated, telomere-based assays of age would make it possible to gain information on: (i) an individual's age via telomere length changes; (ii) species, sex and populational affinities using mtDNA, sex and microsatellite markers (Baker *et al*. 2000); (iii) physiological state via analysis of stress hormones (Wasser *et al*. 2000); (iv) diet through lipid analyses (Olsen & Grahl-Nielsen 2003); and (v) oceanic movement using trace element analyses (Kelly 2000), without lethal sampling.

and avenues in many areas of research, especially in the field of conservation biology (see Box 3). For example, knowledge of biological age in a population of a locally endangered species will be informative in terms of conservation planning (e.g. selecting individuals for introduction and translocation).

Additionally, in a recent paper Stindl (2004) suggests that telomere length of a species may represent 'species age'. He argues that gradual loss of telomere length over thousands of generations may account for many cases of species extinction when telomere length becomes critically short, causing genome instability, and that this same process may result in evolution of new species by reorganizations of chromosomes which are accompanied by subsequent telomere elongation (Stindl 2004). Phylogenetically controlled comparisons of telomere lengths may help to test the validity of these claims.

Conclusions

Although there are some limitations on the use of telomeres in the fields of ecology, evolution and conservation, telomere length (an age estimator) and telomere shortening (an ageing estimator) are poised to become essential tools in molecular ecology, providing immediate access to population age structure without long-term longitudinal studies. Once refined, telomere assays will contribute to answering questions regarding the relationship between reproduction and ageing (or survival) and other aspects of life history at among- and within-species levels. Applications of estimating the biological age of endangered vertebrate populations seem especially promising (Box 3). The use of various techniques that measure telomere length, particularly the complementary use of TRF analysis and Q-PCR, should facilitate telomere research in a variety of species. In most species, research on the ends of chromosomes has yet to start or has just begun, and further data will undoubtedly lead to methodological improvements (e.g. STELA) that will make telomere analysis more accessible. Time will prove whether 'age' and 'ageing' estimations by measuring telomere properties will or will not lead to a paradigm shift in molecular ecology, but we think it likely.

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