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Experimental Gerontology 38 (2003) 791–795

Experimental
Gerontology

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The natural history of telomeres: tools for aging animals and exploring the aging process

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Abstract

We have been exploring the use of telomere length as a technique to age animals. If telomere restriction fragments (TRFs) shorten predictably with age in a particular tissue, then measurement of TRFs will allow estimation of ages of animals when age cannot be measured directly. This would be particularly useful in population studies where tissue samples can be collected, but age of individuals or age structure of the population is otherwise unknown. We have demonstrated that rate of change in length of TRFs from blood cells can be used to estimate age in a number of avian species. Calibration of this telomere ‘clock’ using known-age individuals has led to new questions regarding the importance of TRF shortening in aging and its evolution in animals with differing life spans. Our current data show a tight correlation between telomere rate of change (TROC) and maximum life span in birds, with the longest living species having the slowest TROC. In contrast, absolute length of TRFs is not correlated with maximum life span. Very long-lived Leach’s storm-petrels have telomeres that in fact lengthen with age! These data suggest that in the longest-lived organisms, cellular replicative life span may not be constrained by shortening telomeres. Published data show that TRFs shorten more slowly in long-lived mammals than in short-lived ones, although for birds and mammals of similar life span, telomere shortening is faster in mammals than in birds. This corresponds with the relatively greater longevity in birds than in mammals.

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Keywords: Bird; Mammal; Maximum life span; Senescence; Telomere restriction fragment; Telomere rate of change

1. Introduction

Age of an organism can be critically important in determining its physiological state and response to environmental conditions. Age affects risk of injury and disease, probability of survival and reproductive potential in most species. Age structure is a major determinate of population growth potential. For example, incorporation of age structure into population models of Soay sheep on the St Kilda archipelago increases the model’s power to predict population crashes, because a sheep’s response to weather and density varies with its age (Coulson et al., 2001). Age of humans is usually known from birth records, but that is not the case for most organisms in natural populations. Age can rarely be known with certainty without extensive longitudinal study, and then only for marked individuals or if morphological correlates of age can be established. Correlative techniques (e.g. otolith annuli) may require destructive sampling. Yet knowledge of age can often

provide novel insight into population-level phenomenon that would otherwise be missed.

Because we were interested in the effects of age on reproductive biology in unmarked populations of birds, we set out to determine whether the regular shortening of telomeres from nucleated erythrocytes could be used to estimate age in birds. Telomeres are short tandem repeated sequences of DNA found at the ends of linear eukaryotic chromosomes that, along with associated nucleoproteins, function in stabilizing chromosomal end integrity (Prowse and Greider, 1995). Telomeres in somatic tissue tend to shorten with age, because DNA polymerase is unable to replicate completely the 3’ end of linear DNA. In culture, cells reach a replicative limit after a certain number of cell divisions, which is thought to be due to telomere shortening with each cell replication cycle (Prowse and Greider, 1995). In vivo studies in tissues of a variety of mammals have shown a gradual decrease in telomere length with organismal age (Hausmann et al. in press).

Using telomere length to estimate age of animals first requires determining the rate at which the telomeres shorten in different species (Hausmann and Vleck, 2002). In our efforts to calibrate this ‘telomere clock’ in a variety of birds,

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we discovered that the rate at which telomeres shorten in different species varies with the maximum life span of that species. Long-lived species tend to have telomeres that shorten more slowly, if at all, compared to short-lived species. This suggests that regulation of telomere length in somatic tissues and life span may have co-evolved, so that replicative life span of individual cell lines increases along with longevity of the organism.

2. Methods

We measured the length of telomere restriction fragments (TRFs) in erythrocyte DNA from known-aged individuals of five species of birds (Table 1) and plotted mean telomere length against age to determine the telomere length rate of change (TROC) in each species. The methods used to determine mean TRF length are described in detail in [Hausmann et al. \(2003\)](#).

Each linear chromosome has a single-stranded overhang at each end (G-strand overhang). Our labelled probe binds to this overhang, but avian DNA is known to have substantial interstitial telomeric sequences within the centromere region that can bind the probe if the DNA is single-stranded ([Venkatesan and Price, 1998](#)). When our gels were denatured, these interstitial telomeric sequences appeared. We performed two tests on non-denatured gels to ensure that our probe was binding only to the G-strand overhang of the telomere and not to interstitial repeats. First, before loading some samples on the gel, they were digested with a single-strand specific endonuclease (mung bean nuclease; 40 U) to remove the G-strand overhang. This abolished all binding of the probe, while the same samples not digested with the endonuclease showed

binding of the probe to the G-strand overhang. Second, we hybridized some samples with ^{32}P -labeled $(\text{T}_2\text{AG}_3)_4$ oligonucleotides (the complement of the normal $(\text{C}_3\text{TA}_2)_4$ probe). This would bind to any interstitial telomeric sequences that had somehow become single-stranded, but not to the G-strand overhang. We found no hybridization of the complement probe to our samples, assuring us that the probe was only binding to the G-strand overhang.

3. Results

Length of the TRFs varied systematically with age. TRFs decreased in length with increasing age in the zebra finch, tree swallow, Adélie penguin and common tern, but TRFs increased in length between the youngest individuals and oldest individuals in the Leach's storm petrel (Table 1). The maximum life span (age of oldest individuals living under natural conditions) varies from 5 to more than 35 years in these species, so we summarize these results by plotting mean TRF length of individuals versus relative age, where relative age is calendar age divided by the maximum life span of that species (Fig. 1).

The accuracy with which TRF length can be used to predict age of individuals varies with TROC, the natural variation in TRF among individuals of the same age and the precision with which TRF length can be measured. In all species we have studied, the individuals in the youngest third of those measured can be distinguished from those in the oldest third, and from the middle third as well in all but the zebra finch (Tukey's Honest Significant Difference, $p < 0.05$). In common terns mean telomere length of each age class was significantly different from that of the other age classes ([Hausmann et al., 2003](#)).

The annual TROC in erythrocytes varies with maximum life span such that longer-lived bird species experience less telomere shortening with time than do shorter-lived birds (Fig. 2 ANOVA $F_{1,3} = 262$, $p = 0.0005$, $r^2 = 0.99$). Comparable TROC data are available for eight mammal species, including tissues from spleen, brain, mammary tissue, leukocytes, skin, synovium, fibroblasts and stem cells (see [Hausmann et al., in press](#) for sources). For the mammalian data, we have averaged TROC in all studies that used the same species and plotted the mean values as a function of maximum life spans (Fig. 2). The mammal data are not as linear as the bird data appear to be over the range of life spans for which we have data. There is, however, a significant correlation between maximum life span and TROC in mammals (ANOVA $F_{1,6} = 6.7$, $p < 0.04$, $r^2 = 0.53$); mammals with longer life spans have slower telomere shortening. In addition, there is a significant difference in this relationship between birds and mammals (ANCOVA, $F_{3,9} = 8.3$, $p < 0.01$), such that for animals of the same maximum life span, birds have a slower TROC than do mammals.

Table 1

Comparison of maximum life span, annual rate of change of telomere restriction fragments (mean \pm SE) and predicted lifetime loss of telomere length in erythrocytes in five species of birds. Data from [Hausmann et al. \(in press\)](#)

Species	Maximum life span (years)	Telomere length rate of change (bp/year)	Lifetime telomere loss (kb/lifetime)
Zebra finch (<i>Taeniopygia guttata</i>)	5	-515 ± 95	2.6
Tree Swallow (<i>Tachycineta bicolor</i>)	11	-391 ± 81	4.3
Adélie penguin (<i>Pygoscelis adeliae</i>)	20	-235 ± 48	4.7
Common tern (<i>Sterna hirundo</i>)	26	-57 ± 7	1.5
Leach's Storm-petrel (<i>Oceanodroma leucorhoa</i>)	36	75 ± 10	-2.7^a

^a Note that the Leach's storm-petrel gains telomere repeats throughout life, and to reflect this gain we have indicated lifetime telomere loss as negative.

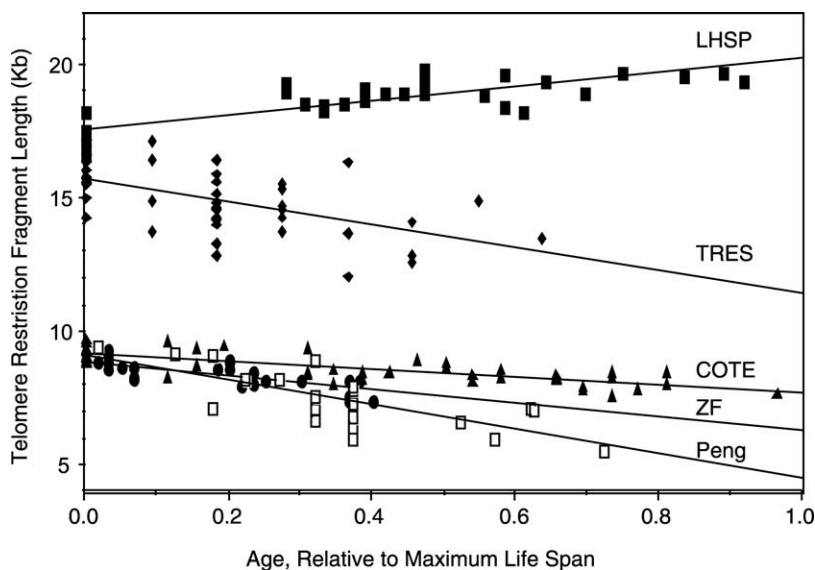


Fig. 1. Length of telomere restriction fragments in known-aged individuals of five bird species: closed boxes, Leach's storm-petrel (LHSP); diamonds, Tree Swallow (TRES); triangles, Common Tern (COTE); circles, zebra finch (ZF); and open boxes, Adélie penguin (Peng). Age is expressed as a fraction of the maximum life span for the species (see Table 1). Data are fit with least-squares regression lines and the slope (TROC) is reported in Table 1.

4. Discussion

A decrease in length of telomeres is an important factor in senescence of replicating cells in culture (Prowse and Greider, 1995), although its role in organismal senescence is much less clear (Rudolph et al., 1999; Cawthon et al., 2003). If telomere shortening does contribute to senescence, there are three different telomere measures that could be important: absolute telomere length at birth, or at senescence (potentially the equivalent of the *critical* telomere

length in cell cultures that have reached replicative senescence) or the rate of change in telomere length.

Our data for birds show no significant relationship between length of telomeres early in life and life span ($p = 0.48$), nor between estimated telomere length in the oldest individuals and life span ($p = 0.25$). Those results come with a caveat, however, because the restriction enzymes we use to isolate TRFs leaves an unknown (but presumably constant) amount of flanking DNA on the TRF. Furthermore, it may not be the *average* telomere length (as we

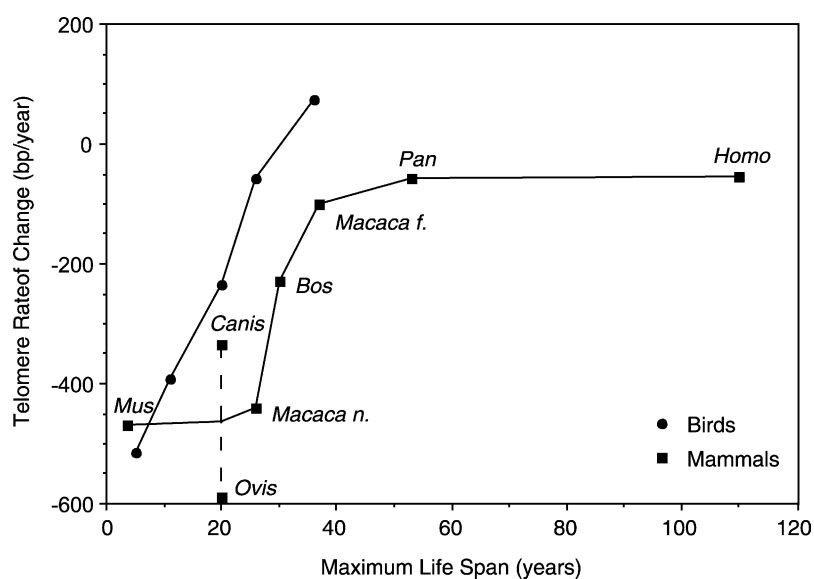


Fig. 2. The relationship between rate of change in mean telomere length (TROC) (base pairs lost [negative numbers] or gained [positive numbers] per year) in various tissues and maximum life span for five birds shown in Table 1 (circles) and eight mammals (boxes). For each class, lines connect the points (or mean of the points for species with the same maximum life span). For mammals (see Haussmann et al. [in press] for sources), common names are: *Mus spretus*, western wild mouse; *Ovis aries*, domesticated sheep; *Canis familiaris*, dog; *Macaca nemestrina*, pigtailed macaque; *Bos taurus*, cattle; *Macaca fascicularis*, cynomolgus monkey; *Pan troglodytes*, chimpanzee; *Homo sapiens*, human.

measure) that is important, but the *shortest* telomere that triggers senescent changes (Hemann et al., 2001). In rodents, as in birds, species differences in mean telomere length are not associated with life span in the few species that have been examined (Hemann and Greider, 2000). Laboratory *Mus musculus* that have been domesticated and inbred for more than 60 years have ultra-long telomeres, but no concomitant increase in life span (Kipling and Cooke, 1990). Other *Mus* and *Peromyscus leucopus* have much shorter TRF lengths, similar to those shown in Fig. 1. In *M. musculus* that lack telomerase due to targeted gene deletion, telomeres shorten with each generation and up to six generations remain viable (Rudolph et al., 1999). Late generation mice with the shortest telomeres do have a shortened life span as well as other signs of premature aging, such as increased incidence of spontaneous malignancies and reduced resistance to wound healing (Rudolph et al., 1999). Delany et al. (2000) used pulsed-field gel electrophoresis to resolve an ultra-long, hypervariable class of TRFs in several species of birds. They suggested this class might be found on the tips of microchromosomes and function to protect critical genes located on these small chromosomes from telomere erosion. Whether the lengths of these ultra-long telomere sequences in birds correlate with life span is not known.

The rate of change in telomere length is correlated with life span in both birds and mammals (Fig. 2), and calibration of this clock allows an estimation of age in animals of unknown age. TROC is lower in long-lived species than in short-lived species. Whether TROC is a determinant or merely a correlate of life span remains to be determined, but if it is the shortest telomere in the cell that signals apoptosis (Hemann et al., 2001), then TROC would affect the length of time before it reaches this critical length.

The mechanisms that could account for adaptive differences in TROC are of considerable interest. These could include a decrease in cell turnover rate, because telomeres shorten only during cell division. Life span of individual erythrocytes does appear to increase with species life span (Rohme, 1981), hence decreasing the number of required cell divisions per lifetime. In germ cells and some stem cell lines, telomeric repeats are maintained by telomerase, a ribonucleoprotein capable of elongating telomeres de novo (Prowse and Greider, 1995). In contrast, most somatic cell lines, except in *M. musculus* and domesticated chickens (Venkatesan and Price, 1998), have low or undetectable telomerase (Prowse and Greider, 1995). Selection for down-regulation of telomerase in somatic tissue may follow from its tumour-promoting tendencies (Rudolph et al., 1999). Up-regulation of telomerase could decrease TROC in long-lived species, but this opens the question of how these species avoid the tumour-susceptibility associated with this mechanism. Finally, decreased rates of oxidative damage might help to decrease telomere

shortening (von Zglinicki et al., 2000). Birds tend to have longer life spans than similar-sized mammals and may also be more resistant to oxidative damage (Ogburn et al., 1998). We are currently pursuing studies to clarify relationships between both telomerase activity and accumulation rate of oxidative damage and life span in various avian species.

The observation that telomeres do not shorten with age, but rather lengthen in long-lived Leach's storm-petrels suggests that complete escape from telomere shortening is possible and may have contributed to this species' remarkable longevity. When such species have achieved life spans through natural selection that are much longer than expected, they should also display a host of correlated characters that are associated with delayed senescence. These natural animal models for longevity deserve more study for strategies and mechanisms they can reveal regarding ways to delay the effects of aging.

Acknowledgements

We thank David Winkler, Katy O'Reilly, Ian Nisbet, Charles Huntington, Judy St Leger and SeaWorld Adventure Park for access to known-aged birds. This study was supported by an Iowa State University Special Research Initiative Grant to CMV and a Glenn/American Federation of Aging Research Scholarship to MFH.

References

- Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenki, A., Kerber, R.A., 2003. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361, 393–395.
- Coulson, T., Catchpole, E.A., Albon, S.D., Morgan, B.J.T., Pemberton, J.M., Clutton-Brock, T.H., Crawley, M.J., Grenfell, B.T., 2001. Age, sex, density, winter weather, and population crashes in soay sheep. *Science* 292, 1528–1531.
- Delany, M.E., Krupkin, A.B., Miller, M.M., 2000. Organization of telomere sequences in birds: evidence for arrays of extreme length and for in vivo shortening. *Cytogenet. Cell Genet.* 90, 139–145.
- Hausmann, M.F., Vleck, C.M., 2002. Telomere length provides a new technique for aging animals. *Oecologia* 130, 325–328.
- Hausmann, M.F., Vleck, C.M., Nisbet, I.C.T., 2003. Calibrating the telomere clock in common terns, *Sterna hirundo*. *Exp. Gerontol.* 38, this issue.
- Hausmann, M.F., Winkler, D.W., O'Reilly, K.M., Huntington, C.E., Nisbet, I.C.T., Vleck, C.M., 2003. Telomeres shorten more slowly in long-lived birds and mammals than in short-lived ones. *Proc. R. Soc. Lond. B. Biol. Sci.* 270, in press.
- Hemann, M.T., Greider, C.W., 2000. Wild-derived inbred mouse strains have short telomeres. *Nucleic Acids Res.* 28, 4474–4478.
- Hemann, M.T., Strong, M.A., Hao, L.-Y., Greider, C.W., 2001. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 107, 67–77.
- Kipling, D., Cooke, H.J., 1990. Hypervariable ultra-long telomeres in mice. *Nature* 347, 400–402.

- Ogburn, C., Austad, S., Holmes, D., Kiklevich, V., Gollahon, K., Rabinovitch, P., Martin, G., 1998. Cultured renal epithelial cells from birds and mice: enhanced resistance of avian cells to oxidative stress and DNA damage. *J. Gerontol. Biol. Sci. Med. Sci.* 53, B287–B292.
- Prowse, K.R., Greider, C.W., 1995. Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc. Natl. Acad. Sci. USA* 92, 4818–4822.
- Rohme, D., 1981. Evidence for a relationship between longevity of mammalian species and life spans of normal fibroblasts in vitro and erythrocytes in vivo. *Proc. Natl. Acad. Sci. USA* 78, 5009–5013.
- Rudolph, K.L., Chang, S., Lee, H.-W., Blasco, M.A., Gottlieb, G.J., Greider, C.W., DePinho, A., 1999. Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* 96, 701–712.
- Venkatesan, R., Price, C., 1998. Telomerase expression in chickens: Constitutive activity in somatic tissues and down-regulation in culture. *Proc. Natl. Acad. Sci. USA* 95, 14763–14768.
- von Zglinicki, T., Pilger, R., Sitte, N., 2000. Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. *Free Radic. Biol. Med.* 28, 64–74.