

Telomerase activity is maintained throughout the lifespan of long-lived birds

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Abstract

Telomerase is an enzyme capable of elongating telomeres, the caps at the ends of chromosomes associated with aging, lifespan and survival. We investigated tissue-level variation in telomerase across different ages in four bird species that vary widely in their life history. Telomerase activity in bone marrow may be associated with the rate of erythrocyte telomere shortening; birds with lower rates of telomere shortening and longer lifespans have higher bone marrow telomerase activity throughout life. Telomerase activity in all of the species appears to be tightly correlated with the proliferative potential of specific organs, and it is also highest in the hatchling age-class, when the proliferative demands of most organs are the highest. This study offers an alternative view to the commonly held hypothesis that telomerase activity is down-regulated in all post-mitotic somatic tissues in long-lived organisms as a tumor-protective mechanism. This highlights the need for more comparative analyses of telomerase, lifespan and the incidence of tumor formation.

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1. Introduction

Telomere dynamics may be one mechanism that shapes life histories (reviewed in Monaghan and Haussmann, 2006). Telomeres, the termini of eukaryotic chromosomes, are composed of short, tandem repeated sequences of DNA. Each time a cell divides, its telomeres shorten due to the inability of DNA polymerase to completely replicate the 3' end of linear DNA. Oxidative damage, caused by free radicals, also causes telomere shortening (von Zglinicki, 2002). Telomeres, therefore, act as a buffer protecting important coding regions of DNA during replication and free radical damage, and this is reflected by ever-shortening telomeres with organismal aging (reviewed in de Magalhães

and Toussaint, 2004; Haussmann et al., 2003). Once telomeres shorten to a critical length, a cell cycle checkpoint is triggered, and cells enter a terminally non-dividing state known as replicative senescence (Hornsby, 2002), which is linked to organismal aging and disease (Cawthon et al., 2003; Miller, 2000; Wu et al., 2003).

Managing telomere loss and repair may be crucial to life history trade-offs. Telomere length regulation is a dynamic process, balancing shortening and maintenance events. The primary mode of telomere restoration is through the enzyme telomerase, a ribonucleoprotein capable of elongating telomeres (Greider and Blackburn, 1985). Telomerase activity is repressed in most normal adult somatic tissue (Forsyth et al., 2002) leading to telomere shortening with increasing age *in vivo* (Taylor and Delany, 2000), and with successive cell division *in vitro* (Bodnar et al., 1998; Counter et al., 1998; Kim et al., 1994). Postnatal

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suppression of telomerase expression in most somatic human tissues is thought to represent a tumor-protective mechanism, preventing immortal growth of transformed cells (Wright and Shay, 2001); more than 90% of all tumors show telomerase activity (Buys, 2000).

Our previous work has shown that erythrocyte telomeres of long-lived bird species shorten at a slower rate than those of short-lived bird species (Haussmann et al., 2003). However, the mechanism underlying the relationship between lifespan and the telomere rate of change (TROC; the average number of base pairs lost or gained each year over the organism's lifespan) is unknown. One possibility is the differential expression of telomerase, whose forced expression in normal human cells extends their proliferative lifespan in culture (Bodnar et al., 1998). Whether extended cellular proliferative lifespan translates into extended organismal lifespan is unknown at this time, although recent evidence supports a link between cellular telomere length and organismal health (Cawthon et al., 2003; Epel et al., 2004; Haussmann et al., 2005; Joeng et al., 2004; Pauliny et al., 2006).

The current study builds on our previous work concerning age-class and tissue differences in telomerase activity in the zebra finch (Haussmann et al., 2004) by expanding these comparisons to three more bird species. Our objectives were: (i) to determine whether telomerase activity is linked with TROC in four bird species with different maximum lifespans, and (ii) to describe telomerase profiles at three ages and in different tissues of four bird species that vary in lifespan.

2. Materials and methods

2.1. Species

All procedures described were approved by the Iowa State University Committee on Animal Care (COAC, 12-02-5346-Q) and are in compliance with the United States of America's laws and policies governing the care and use

of live vertebrate animals in research. All work performed in Canada was approved by the Canadian Wildlife Service (permit number SC2282) and samples were imported into the United States under United States Fish and Wildlife Service permits MB044775-0 and MB058513-1.

We measured telomerase activity in tissues from four species whose maximum lifespans vary widely (Table 1). For each species, we had previously determined the relationship between telomere length and age (measured as TROC, Haussmann et al., 2003). These species include two relatively short-lived species, the zebra finch (*Taeniopygia guttata*) and tree swallow (*Tachycineta bicolor*), and two relatively long-lived species, the common tern (*Sterna hirundo*) and Leach's storm-petrel (*Oceanodroma leucorhoa*). Zebra finches and tree swallows have maximum lifespans similar to those predicted for birds of their size, whereas common terns and Leach's storm-petrels live considerably longer than predicted (Table 1).

Zebra finch samples were collected from a colony maintained at Iowa State University since 1996 (Haussmann and Vleck, 2002). Tree swallow samples were collected from field sites near Ithaca, New York, in a study population followed since 1985 (Winkler and Allen, 1996). Common tern samples were collected on Bird Island, Massachusetts, from a study population followed since 1970 (Nisbet, 2002). Leach's storm-petrel samples were collected on Kent Island, New Brunswick from a population studied since 1947 (Huntington et al., 1996). All birds had been banded as hatchlings and consequently were of precisely known age, except for the oldest common terns, which had been banded as breeding adults and for which the ages listed in Table 1 are minima.

Samples were collected from three age-classes within a species, chosen to represent birds at approximately the same percentage of their species' maximum lifespan, including hatchlings (at 0.08–0.14% of maximum lifespan), young adults close to the age of first breeding (at 9–14% of maximum lifespan) and old adults (at 50–61% of maximum lifespan) (Table 1). For each species, four birds were collected

Table 1

Species studied, maximum observed lifespan in the wild, TROC, and average age of birds sampled for each group (years; means \pm standard deviation), followed by the percent of maximum lifespan for each age-class

Species	Maximum observed lifespan (years) ^a	Predicted maximum lifespan (years) ^b	Lifespan, % of predicted	TROC (bp y ⁻¹) \pm (SE) ^c	Nestling age-class	Young adult age-class	Old adult age-class
Zebra finch ^d	5	7.7	65	-515 \pm 95	0.02 \pm 0.01, 0.14%	0.5 \pm 0.00, 10%	2.5 \pm 0.0, 50%
Tree swallow ^e	11	8.6	128	-391 \pm 65	0.02 \pm 0.00, 0.17%	1.0 \pm 0.00, 9%	6.7 \pm 1.2, 61%
Common tern ^f	29	12.4	233	-57 \pm 7	0.02 \pm 0.01, 0.08%	3.7 \pm 0.58, 13%	>15.0 \pm 1.2, >52% ^h
Leach's storm-petrel ^g	36	10.1	366	75 \pm 10	0.03 \pm 0.01, 0.10%	5.0 \pm 0.00, 14%	22.5 \pm 2.9, 61%

^a Based on published values for each species living in the wild.

^b Predicted lifespan based on body mass from the equation $\log_e \text{lifespan} = 1.514 + 0.21 \log_e M_b$ (Speakman, 2005).

^c Based on values from (Haussmann et al., 2003).

^d Zann (1996).

^e Robertson et al. (1992).

^f Heidinger et al. (2006).

^g Huntington et al. (1996).

^h Reported ages are minima (see text).

from each of the three age-classes, except for old adult tree swallows and young adult common terns (three birds each). Because these are ongoing study populations of free-living birds we were limited in the sample sizes for our destructive sampling.

2.2. Tissue collection and preparations

Birds were euthanized with an overdose of isoflurane. Sex was determined after dissection by gonad type, and so sex was not equalized across age-classes and species. However, both sexes were represented in every age-class of every species. All of the birds collected were in contact with natural parasite and pathogen loads. To assess individual body condition we measured body mass corrected by tarsus length and found that all the birds collected fell within the normal range for their species. All birds were collected during the breeding season and all adults were breeding, another indication that they were in good condition.

The left pectoralis major muscle, the right lobe of the liver, the duodenum from the small intestine, the left kidney, the left lobe of the cerebral cortex, the left gonad, and bone marrow from the femur were collected from tree swallows, common terns and Leach's storm-petrels. Only bone marrow from the femur, the left gonad, the left pectoralis major muscle, and the right lobe of the liver were collected from the zebra finch. Bone marrow was obtained by dissecting the two femurs, removing the epiphyses, and washing out marrow from the diaphysis with a forced stream of phosphate-buffered saline. After dissection, all tissues were perfused with ice-cold PBS to wash free any blood contamination and immediately snap-frozen in liquid nitrogen and then transferred in liquid nitrogen to Iowa State where they were stored at -80°C until use.

Each sample was thawed and a 40–100 mg section of tissue was removed (for bone marrow the entire sample was used), placed into a 1.5 ml microcentrifuge tube containing 200 μl of CHAPS XL lysis buffer and ground into a paste using a plastic pestle. Samples were incubated on ice for 30 min and then centrifuged at 12,000g for 20 min at 4°C . We then transferred 160 μl of the supernatant into a fresh tube and determined the protein concentration by Bradford assay. After protein determination, the remaining tissue lysates were snap-frozen in aliquots and stored at -80°C until use in telomerase assays.

2.3. Telomerase assays

We used a TRAPEze[®] XL telomerase detection kit to measure telomerase activity (Intergen, Gaithersburg, MD). Detection of telomerase activity involves the elongation of a substrate oligonucleotide by telomerase enzyme. Upon completion of PCR amplification, the PCR products fluoresce, and this emission is directly proportional to the amount of telomerase contained in the sample. Because detection of PCR product fluorescence can vary depending

on the instrumentation used, telomerase concentration is expressed in relative fluorescence units (RFU). We made minor modifications to the manufacturer's specifications to allow for optimization of this assay for quantitative PCR (Elmore et al., 2002).

For all tissues, the telomerase repeat amplification protocol (TRAP) assay was completed using a 24 μl reaction mixture containing the substrate oligonucleotide, fluorescent primers and *Taq* DNA polymerase. One microliter of tissue extract (350 ng protein/ μl) was added to the reaction mixture in a 96-well reaction plate. Negative controls included lysis buffer only, lysis buffer without *Taq* polymerase, and a heat-inactivated extract (10 min at 85°C) for each sample. Each sample was run in duplicate along with a heat-inactivated control. If the heat-inactivated control showed telomerase activity the sample was reanalyzed in a subsequent PCR. In all cases, a second run of a heat-inactivated control showed no telomerase activity. A telomerase-positive cell extract (500 cell equivalents) and serial dilutions of a control template oligonucleotide for the creation of a standard curve were also included in each assay. Reaction plates were incubated at 30°C for 30 min and then subjected to real-time PCR using a Stratagene MX3000P[™] (Stratagene). The PCR conditions were 94°C for 3 min followed by 45 cycles of 94°C for 15 s, 59°C for 60 s, and 45°C for 10 s.

Quantification was accomplished by comparing the threshold cycle (C_t ; defined as the initial cycle in which PCR product is detectable) values for each standard and plotting it against telomerase activity to generate a standard curve. All tissues were run in ten assays, with an intra-assay CV of 0.2% and an inter-assay CV of 3.5%, based on a control run in each assay.

2.4. Bone marrow samples

All bone marrow samples had been assayed using end-point PCR, followed by quantification using a fluorescent plate reader as previously reported (Haussmann et al., 2004). Unfortunately we did not have enough bone marrow tissue to re-run these samples using quantitative PCR assay. We have included our previously reported bone marrow telomerase data here for comparison to the other tissue data. While bone marrow telomerase was measured using a different assay, a repeated control that was run in duplicate in both the end-point and quantitative assays gave a low inter-assay CV of 3.9% suggesting the data in the assays are comparable.

2.5. Statistical analysis

The telomerase data were $\log(10) + 3$ transformed for analysis. The constant 3 was introduced to make all reported values positive for ease of interpretation. We used two-way ANOVAs to examine the effects of species and age-class on telomerase activity within each tissue, followed by post hoc Tukey's HSD tests where appropriate. Where F -statistics

returned a significant difference, a power analysis was run before post hoc tests were used. In all cases power was >0.80 . A value of $P < 0.05$ was the criterion for significance.

3. Results

Sample telomerase values ranged from 0 to 4.89 RFUs, and 100 of the 252 samples had no telomerase activity. The telomerase positive control had 5.33 RFUs and the telomerase negative control had 0 RFUs of activity. Table 2 summarizes mean levels of telomerase activity ($\log + 3$ transformed) according to species, age-class and tissue. Formal statistical analysis of the entire data set was not possible because of the small sample sizes ($N = 3\text{--}4$ per group) and the complex pattern of interactions.

3.1. Species, tissue and age-class differences

Averaging over the four tissues that were sampled in all four species and age-classes (bone marrow, gonad, liver, muscle), telomerase activity tended to be highest in Leach's storm-petrels, intermediate in common terns and zebra finches, and lowest in tree swallows (ANOVA, $F_{3,160} = 1.98$, $P = 0.10$) (Fig. 1a). Telomerase activity tended to be higher in hatchlings compared to young and old adults (ANOVA, $F_{2,160} = 2.66$, $P = 0.07$) (Fig. 1b). Averaging over species and age-classes, telomerase activity was highest in bone marrow and gonad, intermediate in

intestine, and lowest in brain, muscle, liver, and kidney ($F_{6,251} = 39.19$, $P = 0.0001$) (Fig. 1c).

3.2. Between species comparisons

3.2.1. Bone marrow

In bone marrow, common terns and Leach's storm-petrels had higher telomerase activity than zebra finches or tree swallows (ANOVA, $F_{3,43} = 9.10$, $P = 0.0002$) (Fig. 2a). Bone marrow telomerase was higher in hatchlings compared to the young and old adults ($F_{2,43} = 7.98$, $P = 0.0015$). Bone marrow telomerase also differed within species at different ages ($F_{6,43} = 10.28$, $P = 0.0009$). Zebra finches and tree swallows had high telomerase activity as hatchlings, but showed a sharp down-regulation in both young and old adults (Tukey HSD, $P < 0.05$), whereas common terns and Leach's storm-petrels had high telomerase activity in bone marrow that did not decrease with age ($P > 0.05$).

3.2.2. Gonad

In the gonad, telomerase activity was detected in all species and each age-class from which gonadal tissue was collected (Fig. 2b). Across all species there was no difference in telomerase activity in testes or ovaries (ANOVA, $F_{1,32} = 1.69$, $P = 0.15$). There was no difference in gonadal telomerase activity among species (ANOVA, $F_{3,32} = 0.55$, $P = 0.47$), age-classes ($F_{2,32} = 0.004$, $P = 0.95$) or within species at different ages (ANOVA, $F_{6,32} = 2.01$, $P = 0.13$).

Table 2

Telomerase activity (relative fluorescence units, $\log_{10} + 3$ transformed): means \pm standard deviation for all species, age-classes and tissues

	Species			
	Zebra finch	Tree swallow	Common tern	Leach's storm-petrel
<i>Hatchling age-class</i>				
Bone marrow	4.71 \pm 0.11	4.48 \pm 0.05	4.57 \pm 0.01	4.36 \pm 0.18
Gonad			3.31 \pm 0.25	3.81 \pm 0.28
Intestine		0.72 \pm 0.34	2.93 \pm 0.66	2.17 \pm 1.25
Brain		1.15 \pm 0.19	2.68 \pm 0.23	4.38 \pm 0.19
Muscle	1.51 \pm 0.57	0.00 \pm 0.00	2.39 \pm 1.23	3.34 \pm 0.13
Liver	1.45 \pm 0.50	0.00 \pm 0.00	0.89 \pm 0.17	2.80 \pm 0.16
Kidney		0.00 \pm 0.00	0.66 \pm 0.66	3.95 \pm 0.28
<i>Young adult age-class</i>				
Bone marrow	3.28 \pm 0.29	3.70 \pm 0.16	4.46 \pm 0.17	4.15 \pm 0.42
Gonad	4.22 \pm 0.09	3.24 \pm 0.30	4.29 \pm 0.14	2.71 \pm 0.93
Intestine		0.00 \pm 0.00	2.80 \pm 1.40	2.99 \pm 1.05
Brain		0.08 \pm 0.08	1.56 \pm 0.36	1.26 \pm 0.74
Muscle	0.00 \pm 0.00	2.09 \pm 1.05	1.04 \pm 0.61	1.22 \pm 0.78
Liver	0.00 \pm 0.00	0.45 \pm 0.45	0.00 \pm 0.00	0.90 \pm 0.62
Kidney		0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
<i>Old adult age-class</i>				
Bone marrow	3.08 \pm 0.35	2.12 \pm 0.89	4.65 \pm 0.13	4.74 \pm 0.07
Gonad	3.69 \pm 0.26	2.75 \pm 0.67	3.98 \pm 0.20	4.12 \pm 0.10
Intestine		0.00 \pm 0.00	4.28 \pm 0.13	3.37 \pm 1.13
Brain		0.00 \pm 0.00	0.23 \pm 0.23	1.04 \pm 0.61
Muscle	0.00 \pm 0.00	1.72 \pm 0.87	0.00 \pm 0.00	0.76 \pm 0.76
Liver	0.97 \pm 0.19	0.12 \pm 0.12	1.01 \pm 0.60	1.74 \pm 0.63
Kidney		0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00

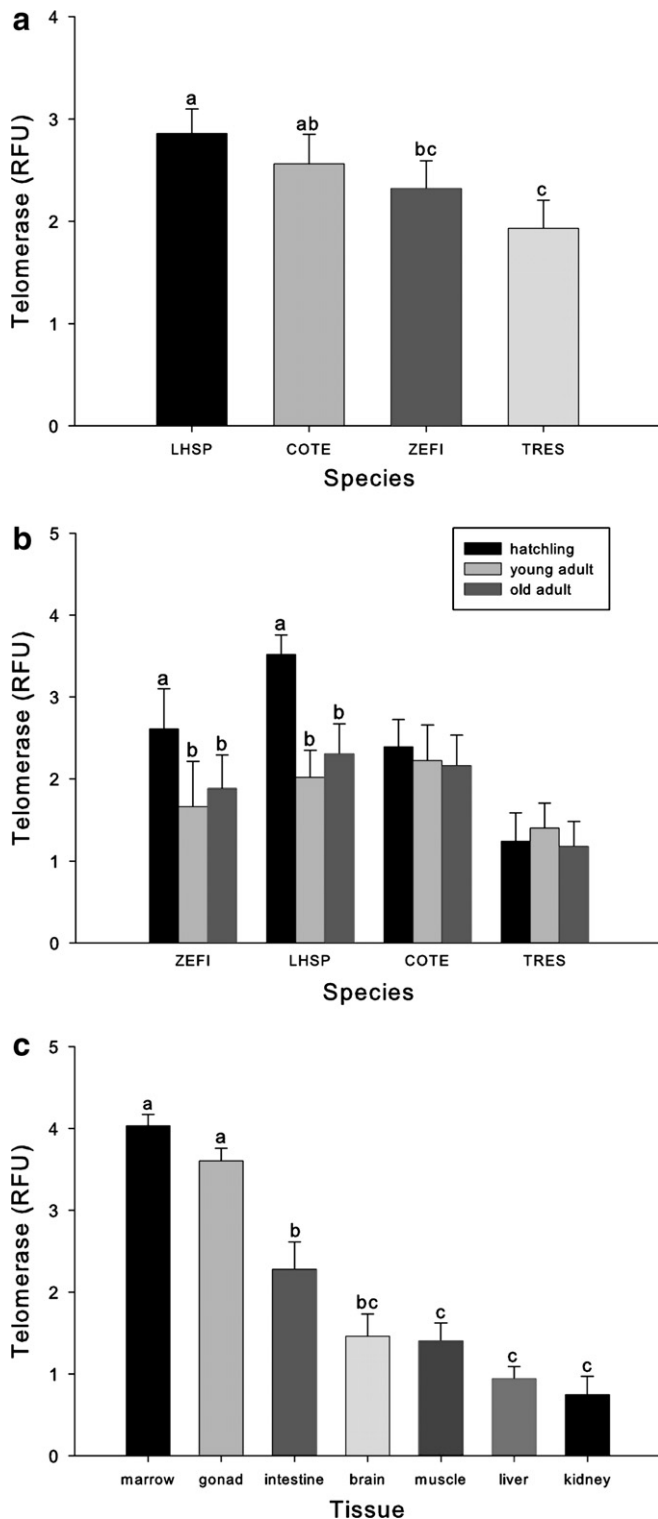


Fig. 1. Telomerase activity (measured in relative fluorescence units): those tissues sampled in all four species (bone marrow, liver, gonad and muscle), (a) averaged over age-classes and tissues, (b) averaged over tissues and species, (c) comparison among all tissues, averaged over all age-classes and species. Plotted values are means (log geometric means + 3) \pm standard deviation. Bars with different letters differ in telomerase activity (Tukey HSD, $P < 0.05$). ZEFI, zebra finch; TRES, tree swallow; COTE, common tern; LHSP, Leach's storm-petrel.

3.2.3. Intestine

In the intestine, telomerase activity was higher in common terns and Leach's storm-petrels than in tree swallows (ANOVA, $F_{2,32} = 11.19$, $P = 0.0004$) (Fig. 2c). There was no difference in telomerase activity among age-classes (ANOVA, $F_{2,32} = 0.52$, $P = 0.60$) or among age-classes within species (ANOVA, $F_{4,32} = 0.58$, $P = 0.68$).

3.2.4. Brain

In the brain, telomerase activity was highest in Leach's storm-petrels, intermediate in common terns and lowest in tree swallows (ANOVA, $F_{2,29} = 31.55$, $P = 0.0001$) (Fig. 2d). Brain telomerase activity was also higher in hatchlings than in young or old adults ($F_{3,29} = 48.71$, $P = 0.0001$). Telomerase activity also differed within species at different ages ($F_{4,29} = 3.26$, $P = 0.04$). Within species, brain telomerase activity was higher in hatchling common tern and Leach's storm-petrel compared to young and old adults (Tukey HSD, $P < 0.05$), but did not vary with age-class in tree swallows ($P > 0.05$).

3.2.5. Kidney

In the kidney, telomerase activity was higher in Leach's storm-petrels than in tree swallows and common terns (ANOVA, $F_{2,27} = 26.41$, $P = 0.0001$) (Fig. 2e). Kidney telomerase activity was higher in hatchlings than in young or old adults ($F_{2,27} = 30.48$, $P = 0.0001$), and also differed among age-classes within species ($F_{4,27} = 24.08$, $P = 0.0001$). Within species, kidney telomerase activity was higher in Leach's storm-petrel hatchlings than in young and old adults (Tukey HSD, $P < 0.05$), but did not vary with age-class in tree swallows or common terns ($P > 0.05$).

3.2.6. Skeletal muscle

In skeletal muscle, telomerase activity did not differ among species (ANOVA, $F_{3,38} = 0.85$, $P = 0.44$), but did vary by age-class; hatchlings having higher telomerase activity than young or old adults ($F_{2,38} = 6.93$, $P = 0.01$) (Fig. 2f). Muscle telomerase activity also differed among age-classes within species ($F_{2,38} = 3.30$, $P = 0.02$). Zebra finches had higher muscle telomerase in hatchlings than in young and old adults (Tukey HSD, $P < 0.05$), but telomerase activity did not vary with age-class in the other three species ($P > 0.05$).

3.2.7. Liver

In the liver, telomerase activity was higher in Leach's storm-petrels than in the other three species (ANOVA, $F_{3,44} = 10.40$, $P = 0.0001$) (Fig. 2g). Liver telomerase activity was highest in hatchlings, intermediate in old adults and lowest in young adults ($F_{3,44} = 3.21$, $P = 0.05$). Liver telomerase activity also differed among age-classes within species ($F_{6,44} = 2.81$, $P = 0.03$), being lower in young adults than in hatchlings or old adults in the zebra finches, common terns and Leach's storm-petrels (Tukey HSD, $P < 0.05$), but did not vary with age-class in the tree swallow ($P > 0.05$).

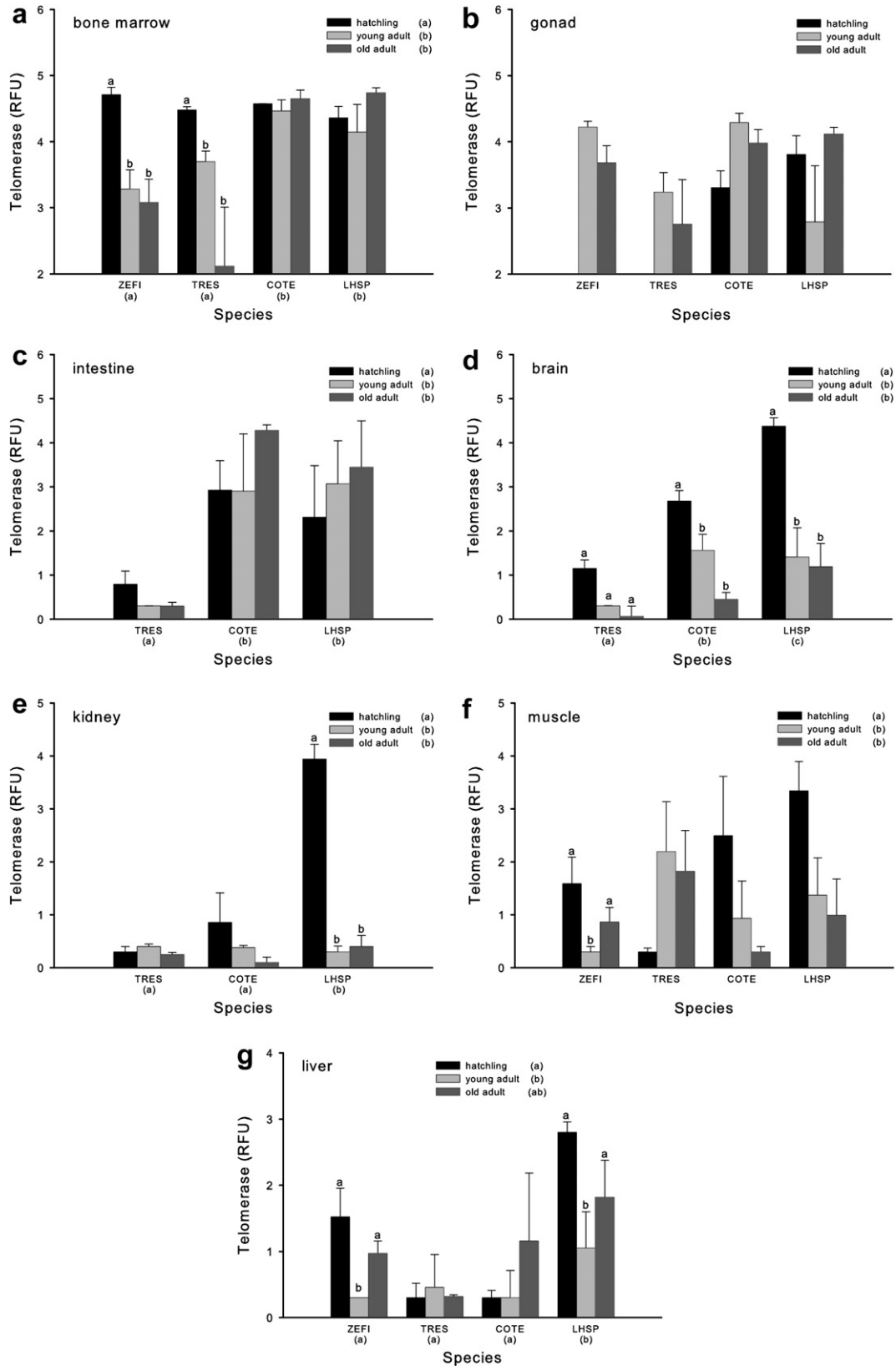


Fig. 2. Telomerase activity (measured in relative fluorescence units) in hatchling, young adult and old adult zebra finches (ZEFI), tree swallows (TRES), common terns (COTE), and Leach's storm-petrels (LHSP) in (a) bone marrow, (b) gonads, (c) intestine, (d) brain, (e) kidney, (f) muscle, and (g) liver. Plotted values are means (log geometric means + 3) \pm standard deviations. Differences among species (averaged over age-classes) are denoted by different letters on the x-axis. Differences among age-classes (averaged over species) are denoted by different letters in the legend. Differences among age-classes within species are denoted by different letters above bars.

4. Discussion

4.1. Telomerase activity is associated with telomere rate of change

Telomere stability and regulation of telomerase activity have been directly linked to *in vitro* cellular senescence (Bodnar et al., 1998), and more recently to *in vivo* aging and disease (Cawthon et al., 2003; Wu et al., 2003). Our previous work showed that regulation of telomere dynamics in erythrocytes is associated with lifespan (Haussmann et al., 2003). The hematopoietic stem cells of the bone marrow produce all circulating erythrocytes in birds (Sturkie and Griminger, 1976), and telomere lengths measured in circulating blood cells are representative of stem cell telomere lengths in bone marrow (Vaziri et al., 1994). We found that telomerase profiles in the bone marrow of the four avian species differ between the short- and long-lived species. The short-lived zebra finch and tree swallow sharply down-regulate bone marrow telomerase before adulthood, whereas the long-lived common tern and Leach's storm-petrel express bone marrow telomerase at high levels throughout life which can account for the slower rates of erythrocyte telomere shortening in the longer-lived birds. Other factors, such as DNA damage susceptibility, DNA repair capabilities, and shelterin proteins which controls the synthesis of telomeric DNA by telomerase (de Lange, 2006) also are likely to be important in regulating TROC in species. Whether natural selection has acted on telomerase or these other mechanisms to adjust the rate at which telomeres shorten and thereby modified lifespan awaits further comparative study.

4.2. Telomerase activity and lifespan

Current scientific thought holds that long-lived organisms down-regulate telomerase at early developmental stages as a tumor-protective mechanism, because telomerase activity allows for unlimited cellular proliferation (Buys, 2000; Campisi et al., 2001; Djojotubroto et al., 2003; Wright and Shay, 2001). Conversely, it has been suggested that species with short lifespans do not possess a mechanism to down-regulate telomerase for tumor-protection. For example, short-lived laboratory mice express telomerase in many tissues throughout adulthood (Coviello-McLaughlin and Prowse, 1997; Prowse and Greider, 1995) and have a high incidence of cancer. However, these theories have been developed primarily from research on humans (reviewed in Forsyth et al., 2002) and laboratory mice. Telomerase activity in other domesticated animals has shown no clear pattern with lifespan, as some species have telomerase profiles more similar to humans (horses, Argyle et al., 2003; sheep, Cui et al., 2003; domestic cats, McKeivitt et al., 2003; domestic dogs, Nasir et al., 2001), and other species have telomerase profiles more similar to laboratory mice (pigs, Fradiani et al., 2004; domestic cats, Yazawa et al., 2003).

Our data appear inconsistent with the hypothesis that telomerase regulation varies with species lifespan as a tumor-protective mechanism. We found that the long-lived species were not more likely to down-regulate telomerase at early developmental stages compared to the short-lived species. On the contrary, the Leach's storm-petrel and common tern had higher overall telomerase activity in the tissues we measured than the other two species. High telomerase activity in Leach's storm-petrels in general was primarily due to very high telomerase activity in tissues at the hatchling age-class. However, even when the data from the hatchling age-class were excluded from the analysis, these birds still had higher telomerase activity throughout life than did the zebra finches or tree swallows (ANOVA, $F_{3,175} = 7.98$, $P = 0.0001$).

Our finding that long-lived birds such as common terns and Leach's storm-petrels maintain telomerase in some tissues throughout life is intriguing in view of the association between telomerase, cell proliferation and cancer in mammals. Studies report a low incidence of cancer in wild birds, and specifically a very low incidence in long-lived seabirds (Effron et al., 1977; Siegfried, 1983, M. Pokras, personal communication). This may reflect the lack of focused study of long-lived individuals, but more investigation of this topic is needed.

4.3. Telomerase activity and age

In examining telomerase levels in our four study species, we found evidence for a general down-regulation of telomerase after the hatchling age-class. Specifically, hatchling birds had higher telomerase in the bone marrow, brain, kidney, skeletal muscle and liver. In most tissues and species, there was no difference in telomerase levels between young and old adults. Down-regulation of telomerase during developmental progression in proliferation-competent cells is common and occurs in chickens (Taylor and Delany, 2000; Venkatesan and Price, 1998) and humans (Wright et al., 1996). High telomerase activity early in development is thought to reflect the greater proliferative demands of most tissues at this time (Forsyth et al., 2002), and telomerase is rapidly reduced in terminally differentiated cells in a post-mitotic state (Greenberg et al., 1998).

The four species in this study also differ in their developmental trajectories. Common terns and Leach's storm-petrels are classified as semiprecocial species and zebra finches and tree swallows are classified as altricial species (Starck and Ricklefs, 1998). Based on this scale one might predict that the tern and storm-petrel are more developed at hatching than the finch or swallow, necessitating less cell proliferation and lower telomerase expression. However, we found that in hatchlings, telomerase activity was much higher in most tissues of the semiprecocial birds than in the altricial ones. This suggests that developmental mode may not reflect tissue growth and cell division necessitating elevated telomerase activity.

4.4. Telomerase activity varies among tissues

The tissues we analyzed for telomerase activity can be classified broadly into two groups. Once adult size has been reached, some tissues are largely post-mitotic, not requiring much cell division, and therefore do not require telomerase activity to maintain telomere length (Delany et al., 2003). Examples of these tissues are brain (Klapper et al., 2001), kidney (Mueller et al., 1999), skeletal muscle (Wernig et al., 2005) and liver (Rhyu, 1995). Other tissues, such as bone marrow (Broccoli et al., 1995), gonads (Prowse and Greider, 1995; Taylor and Delany, 2000), and intestine (Harley et al., 1994; Hiyama et al., 1996), contain a large component of stem cells, which proliferate throughout life and may require active telomere maintenance through telomerase expression (Forsyth et al., 2002). Across our four species and all age-classes, telomerase activity was generally higher in the proliferative tissues than in the post-mitotic tissues. Post-natally, telomerase activity was largely absent in the brain, kidney, skeletal muscle and liver in all species. These profiles are generally comparable to those found in chickens (Taylor and Delany, 2000; Venkatesan and Price, 1998), although we found higher telomerase activity in the skeletal muscle, kidney and brain of hatching common terns and Leach's storm-petrels than is found reported in chickens.

Telomerase profiles in the bone marrow, gonads and intestine were elevated at all stages of life. Telomerase activity in the proliferative somatic tissues of humans is confined to specific cell subpopulations (Meeker and Coffey, 1997). We did not subdivide our tissues by cell type before analysis and the high telomerase activity we measured is likely due to stem cells in the bone marrow (Broccoli et al., 1995) and intestine (Hiyama et al., 1996), and germ cells in the gonads (reviewed in Forsyth et al., 2002; Prowse and Greider, 1995).

5. Conclusions

This study builds on existing knowledge of levels of telomerase activity and changes across lifespan by including analyses of wild bird species that vary greatly in their life histories. The positive correlation between TROC and lifespan in bird erythrocytes may be mediated by differential telomerase expression in the bone marrow of the short and long-lived species. While our data are generally in accordance with the findings of other studies on which tissues possess telomerase activity and when telomerase is down-regulated, it suggests an alternative to the view that telomerase is down-regulated early in all long-lived species as a tumor-protective mechanism.

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References

- Argyle, D., Ellsmore, V., Gault, E.A., Munro, A.F., Nasir, L., 2003. Equine telomeres and telomerase in cellular immortalisation and ageing. *Mech. Ageing Dev.* 124, 759–764.
- Bodnar, A.G., Ouellette, M., Frolkis, M., Holt, S.E., Chiu, C.P., Morin, G.B., Harley, C.B., Shay, J.W., Lichtsteiner, S., Wright, W.E., 1998. Extension of life-span by introduction of telomerase into normal human cells. *Science* 279, 349–352.
- Broccoli, D., Young, J.W., deLange, T., 1995. Telomerase activity in normal and malignant hematopoietic cells. *Proc. Natl. Acad. Sci. USA* 92, 9082–9086.
- Buys, C.H.C.M., 2000. Telomeres, telomerase, and cancer. *N. Engl. J. Med.* 342, 1282–1283.
- Campisi, J., Kim, S.H., Lim, C.S., Rubio, M., 2001. Cellular senescence, cancer and aging: the telomere connection. *Exp. Gerontol.* 36, 1619–1637.
- Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., Kerber, R.A., 2003. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 361, 393–395.
- Counter, C.M., Hahn, W.C., Wei, W.Y., Caddle, S.D., Beijersbergen, R.L., Lansdorp, P.M., Sedivy, J.M., Weinberg, R.A., 1998. Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. *Proc. Natl. Acad. Sci. USA* 95, 14723–14728.
- Coviello-McLaughlin, G.M., Prowse, K.R., 1997. Telomere length regulation during postnatal development and ageing in *Mus spretus*. *Nucleic Acids Res.* 25, 3051–3058.
- Cui, W., Wylie, D., Aslam, S., Dinnyes, A., King, T., Wilmut, I., Clark, A.J., 2003. Telomerase-immortalized sheep fibroblasts can be reprogrammed by nuclear transfer to undergo early development. *Biol. Reprod.* 69, 15–21.
- de Lange, T., 2006. Mammalian Telomeres. second ed., 387–431.
- de Magalhães, J.P., Toussaint, O., 2004. Telomeres and telomerase: a modern fountain of youth? *Rejuvenation Res.* 7, 126–133.
- Delany, M.E., Daniels, L.M., Swanberg, S.E., Taylor, H.A., 2003. Telomeres in the chicken: genome stability and chromosome ends. *Poult. Sci.* 82, 917–926.
- Djojotubroto, M.W., Choi, Y.S., Lee, H.W., Rudolph, K.L., 2003. Telomeres and telomerase in aging, regeneration and cancer. *Mol. Cell* 15, 164–175.
- Effron, M., Griner, L., Benirschke, K., 1977. Nature and rate of neoplasia found in captive wild mammals, birds, and reptiles at necropsy. *J. Natl. Cancer Inst.* 59, 185–198.
- Elmore, L.W., Forsythe, H.L., Ferreira-Gonzalez, A., Garrett, C.T., Clark, G.M., Holt, S.E., 2002. Real-time quantitative analysis of telomerase activity in breast tumor specimens using a highly specific and sensitive fluorescent-based assay. *Diagn. Mol. Pathol.* 11, 177–185.
- Epel, E.S., Blackburn, E.H., Lin, J., Dhabhar, F.S., Adler, N.E., Morrow, J.D., Cawthon, R.M., 2004. Accelerated telomere shortening in response to life stress. *Proc. Natl. Acad. Sci. USA* 101, 17312–17315.
- Forsyth, N.R., Wright, W.E., Shay, J.W., 2002. Telomerase and differentiation in multicellular organisms: turn it off, turn it on, and turn it off again. *Differentiation* 69, 188–197.
- Fradiani, P.A., Ascenzioni, E., Lavitrano, M., Donini, P., 2004. Telomeres and telomerase activity in pig tissues. *Biochimie* 86, 7–12.

- Greenberg, R.A., Allsopp, R.C., Chin, L., Morin, G.B., DePinto, R.A., 1998. Expression of mouse telomerase reverse transcriptase during development, differentiation and proliferation. *Oncogene* 16, 1723–1730.
- Greider, C.W., Blackburn, E., 1985. Identification of a specific telomere terminal transferase activity in *Tertahymena* extracts. *Cell* 43, 405–413.
- Harley, C.B., Kim, N.W., Prowse, K.R., Weinrich, S.L., Hirsch, K.S., West, M.D., Bacchetti, S., Hirte, H.W., Counter, C.M., Greider, C.W., Wright, W.E., Shay, J.W., 1994. Telomerase, cell immortality, and cancer. *Cold Spring Harbor Symp. Quant. Biol.* 59, 307–315.
- Haussmann, M.F., Vleck, C.M., 2002. Telomere length provides a new technique for aging animals. *Oecologia* 130, 325–328.
- Haussmann, M.F., Winkler, D.W., Huntington, C.E., Nisbet, I.C.T., Vleck, C.M., 2004. Telomerase expression is differentially regulated in birds of differing life span. Strategies for engineered negligible senescence: why genuine control of aging may be foreseeable. *Ann. N. Y. Acad. Sci.* 1019, 186–190.
- Haussmann, 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, 1387–1392.
- Haussmann, M.F., Winkler, D.W., Vleck, C.M., 2005. Longer telomeres associated with higher survival in birds. *Biol. Lett.* 1, 212–214.
- Heidinger, B.J., Nisbet, I.C.T., Ketterson, E.D., 2006. Older parents are less responsive to a stressor in a long-lived seabird: a mechanism for increased reproductive performance with age? *Proc. R. Soc. Lond. B Biol. Sci.* 273, 2227–2231.
- Hiyama, E., Hiyama, K., Tatsumoto, N., Kodama, T., Shay, J.W., Yokoyama, T., 1996. Telomerase activity in human intestine. *Int. J. Oncol.* 9, 453–458.
- Hornsby, P.J., 2002. Cellular senescence and tissue aging *in vivo*. *J. Gerontol.* 57A, B251–B256.
- Huntington, C.E., Butler, R.G., Mauck, R.A., 1996. Leach's storm-petrel. 1–32.
- Joeng, K.S., Song, E.J., Lee, K.J., Lee, J., 2004. Long lifespan in worms with long telomeric DNA. *Nat. Genet.* 36, 607–611.
- Kim, N.W., Piatyszek, M.A., Prowse, K.R., Harley, C.B., West, M.D., Ho, P.L.C., Coviello, G.M., Wright, W.E., Weinrich, S.L., Shay, J.W., 1994. Specific association of human telomerase activity with immortal cells and cancer. *Science* 266, 2011–2015.
- Klapper, W., Shin, T., Mattson, M.P., 2001. Differential regulation of telomerase activity and TERT expression during brain development in mice. *J. Neurosci. Res.* 64, 252–260.
- McKevitt, T.R., Nasir, L., Wallis, C.V., Argyle, D.J., 2003. A cohort study of telomere and telomerase biology in cats. *Am. J. Vet. Res.* 64, 1496–1499.
- Meeke, A.K., Coffey, D.S., 1997. Telomerase: a promising marker of biological immortality of germ, stem, and cancer cells. A review. *Biochemistry (Mosc.)* 62, 1323–1331.
- Miller, R.A., 2000. Telomere diminution as a cause of immune failure in old age: an unfashionable demerit. *Biochem. Soc. Trans.* 29, 241–245.
- Monaghan, P., Haussmann, M.F., 2006. Do telomere dynamics link lifestyle and lifespan? *Trends Ecol. Evol.* 21, 47–53.
- Mueller, M., Heicappel, R., Krause, H., Sachsinger, J., Porsche, C., Miller, K., 1999. Telomerase activity in malignant and benign renal tumors. *Eur. Urol.* 35, 249–255.
- Nasir, L., Devlin, P., McKevitt, T., Rutteman, G., Argyle, D.J., 2001. Telomere lengths and telomerase activity in dog tissues: a potential model system to study human telomere and telomerase biology. *Neoplasia* 3, 351–359.
- Nisbet, I.C.T., 2002. Common Tern. 1–40.
- Pauliny, A., Wagner, R.H., Augustin, J., Szep, T., Blomqvist, D., 2006. Age-independent telomere length predicts fitness in two bird species. *Mol. Ecol.* 15, 1681–1687.
- 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.
- Rhyu, M.S., 1995. Telomeres, telomerase, and immortality. *J. Natl. Cancer Inst.* 87, 884–894.
- Robertson, R.J., Stutchbury, B.J., Cohen, R.R., 1992. Tree Swallow. 1–28.
- Siegfried, M., 1983. Neoplasms identified in free-living birds. *Avian Dis.* 27, 86–99.
- Speakman, J.R., 2005. Body size, energy metabolism and lifespan. *J. Exp. Biol.* 208, 1717–1730.
- Starck, J.M., Ricklefs, R.E., 1998. Patterns of development: the altricial-precocial spectrum. In: Strack, J.M., Ricklefs, R.E. (Eds.), *Avian Growth and Development: Evolution within the Altricial-Precocial Spectrum*. Oxford University Press, Oxford, pp. 3–30.
- Sturkie, P.D., Griminger, P., 1976. Blood: physical characteristics, formed elements, hemoglobin, and coagulation. In: Sturkie, P.D. (Ed.), *Avian Physiology*. Springer-Verlag, New York, pp. 53–75.
- Taylor, H.A., Delany, M.E., 2000. Ontogeny of telomerase in chicken: impact of downregulation on pre- and postnatal telomere length *in vivo*. *Dev. Growth Differ.* 42, 613–621.
- Vaziri, H., Dragowska, W., Allsopp, R.C., Thomas, T.E., Harley, C.B., Lansdorp, P.M., 1994. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc. Natl. Acad. Sci. USA* 91, 9857–9860.
- Venkatesan, R.N., 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., 2002. Oxidative stress shortens telomeres. *Trends Biochem. Sci.* 27, 339–344.
- Wernig, A., Schäfer, R., Knauf, U., Mundegar, R.R., Zweyer, M., Högemeier, O., Martens, U.M., Zimmermann, S., 2005. On the regenerative capacity of human skeletal muscle. *Artif. Organs* 29, 192–198.
- Winkler, D.W., Allen, P.E., 1996. The seasonal decline in tree swallow clutch size: physiological constraint or strategic adjustment? *Ecology* 77, 922–932.
- Wright, W.E., Piatyszek, M.A., Rainey, W.E., Byrd, W., Shay, J.W., 1996. Telomerase activity in human germline and embryonic tissues and cells. *Dev. Genet.* 18, 173–179.
- Wright, W.E., Shay, J.W., 2001. Cellular senescence as a tumor-protection mechanism: the essential role of counting. *Curr. Opin. Genet. Dev.* 11, 98–103.
- Wu, X.F., Amos, C.I., Zhu, Y., Zhao, H., Grossman, B.H., Shay, J.W., Luo, S., Hong, W.K., Spitz, M.R., 2003. Telomere dysfunction: a potential cancer predisposition factor. *J. Natl. Cancer Inst.* 95, 1211–1218.
- Yazawa, M., Okuda, M., Uyama, R., Nakagawa, T., Kanaya, N., Nishimura, R., Sasaki, N., Masuda, K., Ohno, K., Tsujimoto, H., 2003. Molecular cloning of the feline telomerase reverse transcriptase (TERT) gene and its expression in cell lines and normal tissues. *J. Vet. Med. Sci.* 65, 573–577.
- Zann, R.A., 1996. *The Zebra Finch: A Synthesis of Field and Laboratory Studies*. Oxford University Press, Oxford.